ME/CFS Symposium – May 2, 2024

Transcript

Dr. Avindra Nath: My name is Dr. Avi Nath, and I'm the clinical director of the National Institute of Neurological Disorders and Stroke. I wanted to welcome all of you here, and the opening remarks will be given by Dr. Walter Koroshetz who is the director of the National Institute of Neurological Disorders and Stroke and has been a very strong supporter of research in ME/CFS over the years. Without his help, this would not be possible at all. Thank you, Dr. Walter Koroshetz for coming and addressing us. Do you have slides?

Dr. Walter Koroshetz: Thank you to everyone who is here, thank you to the people listening in. As Avi mentioned, I'm the director at NINDS. We have been working with our other institutes, primarily NIAID, and Joe's here from NIAID for a number of decades to try to understand the biology of ME/CFS and develop treatments.

I think we all shared the general frustration around the world, that this is going way too slow, there is too much suffering going on out there and we don't have answers for people. So, I am really proud of the work that was done here by Dr. Brian Walitt, Avi Nath, and the whole team, which we will hear from today to try to take an intense look at people who had ME/CFS using the resources that are here at the Clinical Center, that are really unique. So they really took advantage of that and I hope that folks can get a sense of what they found to try to put them into the right context.

I would say that this article that came out saying that we finally know what drives chronic fatigue syndrome along with Long COVID is probably an overstatement. I think you could bet heavily it's an overstatement. I think the things you'll be hearing about today and the small number of patients are very intriguing, they have been very clearly rigorously studied, and the data is as pristine as you can get. There is a lot of questions that are going to arise from this, and how it all pans out in the end. I think is what we will help stimulate research around the country and around the world to get at the bottom of this problem.

There's a lot more interest in this problem now, and probably a lot of that comes from the fact that ME/CFS is a major chronic illness that occurs after COVID infections, which occurred in millions and hundreds of millions of people around the world. So, the problem has just been magnified and unfortunately, our ignorance has also magnified.

There was a recent National Academy report coming out on the common research agenda for infection associated chronic illnesses, the hope is that this will galvanize the scientific community which has really been reticent to study ME/CFS to make a difference.

Also, I would say that we are going to be talking in neurological terms a lot of the time here, and I think that that may be something new to people and I'm going to talk a little bit about terminology in a second. Just to say that the biology of fatigue is really poorly understood as a general concept, and is something that is inherently important in everything we do, every second, every microsecond, there is a calculation going on in our brain about what is the reward effort match in trying to understand the biology of fatigue which occurs in so many different conditions was the subject of the group coming out of the Blueprint, they published this paper in Sleep Research Society.

There is clearly, as I mentioned, a bigger emphasis on this problem given the COVID pandemic and ME/CFS coming on as a chronic complication of COVID. There is work going on to unravel these two conditions simultaneously. There is certainly may be some differences, but we suspect there is way more in common than there are differences. Of note, the NINDS has been working over the past year, Vicky Whittemore is here, who kind of led this to develop a roadmap, a research roadmap for ME/CFS research that we hope will also be able to

give some guidance to scientists around the world on what the current state of affairs are, and what things look like that are worth moving into. I would say a lot of the results you'll hear about today came after that so what you're going to be hearing about is additional stuff.

I wanted to say that the effort here, that Avi Nath and Brian Walitt have been working on is looking at this general problem, so they have looked and you'll hear about the ME/CFS study, and we have also studied Long COVID patients with similar testing to be able to compare results and they also have a project going on with the Veterans Administration looking at Gulf War illness in which there is significant overlap in issues.

The Roadmap, that's going to presented at our Council coming up at the end of May, and that's during an open session of our NINDS Council, people please zoom in for that. They are looking at a whole bunch of different systems, and data coming out of all different countries, different types of labs and trying to synthesize best they can but putting it all together has really been a problem. Hopefully, this is also going to stimulate more work in the area.

They have looked at multiple webinars to develop the Roadmap in all these different systems and came up with targets to look at as potential drivers.

In terms of COVID, I just have here a slide, this is the report of the symptoms that are associated with Long COVID coming out very early after the pandemic or actually, during the pandemic. You can see how, in yellow, what the overlap is in many of the symptoms of ME/CFS. And actually, post-exertional malaise is a symptom in Long COVID patients that is the most specific for Long COVID. So, there's a lot of overlap here.

In the RECOVER Initiative, which is very well-financed to study people with Long COVID, we're looking, trying to understand the pathobiology, doing clinical trials, we don't have a lot of clues as to what is causing it yet but we are following up on even the partial clues that we have and also trying to try different symptomatic therapies, which if they are effective in Long COVID, one certainly would think it would be tried in people with ME/CFS outside of Long COVID.

A lot of work going on here, maybe also more important than anything, is there is thousands of people working on this problem. What we need to do is to have these folks realize the commonalities between these different conditions as Dr. Nath and his team have all along, and that would be really great moving into the future, to have this kind of army working on the problem most important to people with ME/CFS.

I wanted to spend a little of time on how neurologists think. We are peculiar people, you will find that out more today. We try to think about how the brain works and there is a neurology to everything. There is a neurology to why this guy here is moving his legs back and forth, there is a neurology to why you are smiling, and you are smiling. It's everything we do is through the circuits in the brain. In ME/CFS, these circuits are not functional. We are trying to find out what is the problem with these circuits.

This is an area which is so primitive to any organism but is so understudied, we know a lot about why we eat, when we don't eat, feel satiety, we even have drugs now that interfere in this system to produce weight loss. Making an effort to do something is as primitive as eating, and there are circuits in the brain that are devoted to everything we do, including the decisions to make an effort on anything that we do, everything.

Now the problem is, the word "effort" has connotations in the lay press that don't apply to what we are doing as neurologists. What we are trying to think about is how the brain is making this decision, what am I going to do on a microsecond level, this is not like I'm going to decide to make an effort or not, this is what is going on in your brain constantly, constantly, constantly, and you don't even know what is going on. It's not in your consciousness, you couldn't think about every single motion that you do and decide in a deliberate way, am I going to make that motion. You could make maybe 100 a day and I'm making 100 in three seconds.

So then the question is, what is fatigue? When we think about different ways, I'm not an expert, Dr. Hallett is an expert until talk to you later but what I think about it is, your brain is trying to make a decision, in a very simplistic way is I'm going to do X for reward Y. That is every single action you make is, your brain is making that computation, so you have to make some estimation of what the work or the effort is, and then you make some estimation of what the reward is and your brain is doing that all the time.

Then fatigue, there is a real problem there. We are not exactly sure what the problem is, it could be that you are overestimating the effort, and your brain is overestimating the effort or it could be that you're not getting the reward signal for the effort. But that is a computational problem in your brain.

It's really important that people understand this, it has nothing to do with they don't want to make an effort, it has nothing to do with that. It's completely separate. It's how your brain is making these computations.

Just an example, so if you look at muscle fatigue, here is really something simple and nothing to do with ME/CFS, it's about fatigue. When you try to make contractions against the force, eventually you will fatigue and you see here, what happens is that the force starts to drop off after you do it 100 times. The interesting thing is, you say okay, the muscle can't do it anymore. Not true because when you stimulate the nerve come you get the contractions to come back. There is something in the central nervous system that is telling the nerves, I don't want to do this anymore, you stop firing. There's nothing wrong with the muscle unless it is tired, and it can't get all the way up to what it did before but look at the big difference you get when you stimulate it directly.

That is one sense of fatigue that there is evidence that this is coming from a central controller That is what I think we are really trying to understand with ME/CFS, where is that central controller and what is wrong with it. There's a lot of things that will cause you to be fatigued, that's not the problem, we know a lot of what the list of the causes are, and we can inject you with something that will give you fatigue. How that's happening in the brain is what the big question is, and why people with ME/CFS, they have that reset and balance being, why is it abnormal.

I would like people to just try to put their lay terminology aside for a second and really listen carefully to what the scientists are going to tell you today. It's the science that is going to give us the answers, once we understand what is going on in the brain, we can find out where in the brain is occurring and now with BRAIN Initiative technology, you can modulate circuits. So we have people who have severe chronic pain, you stimulate a certain area and it goes away. People with depression, close to suicide, you turn on the stimulator and it goes away.

So, there is a real promise and understanding what is wrong with these circuits and that is what you will hear about today from Dr. Nath and the team, and hopefully this moves us a step further to better understanding of treatments for ME/CFS.

I really appreciate all the work that has been done, a tremendous amount of work that has been done and I really think it's a credit to Avi and his team to make this public and try to explain it in the best way possible to the world. Thanks very much.

Dr. Avindra Nath: Thank you, Dr. Koroshetz. That was a very elegant description, a lot better than I could have ever explained myself. Thank you.

Okay, I'm just going to give a few opening remarks just to put this all in context. The first thing I want to do is really tell you where all of this research took place. You're in Building 10 right now, these three buildings together that you see are the Clinical Research Center and that is where all the patients came, it's the largest research hospital in the world, but yet it's built as a 200-bed hospital and only 90 occupied at the moment.

It is although large for research, small in context. It gives us the ability to really study patients in a way that you never can otherwise. My office and lab is right within Building 10 itself, so it provides the ease of being able to see the patients and the research all in the same place, you don't have to get in your car to go to another place.

I want to thank a lot of people who made this possible. First of all, I really want to thank the study participants, because they really spent a lot of time and effort coming here knowing that there is no tangible benefit to them at the end of this study. But yet, we probed them and did all kinds of tests to them, took blood and spinal fluid, imaging, and all kinds of things, and they went through all of it. They were here for a week and then two weeks. We are extremely grateful to them because without their volunteering this would not be possible.

The study participants are not just the patients with ME/CFS but healthy controls who came and went to the same process as well. So they definitely are not benefiting in any way at all but they were willing to put themselves through it in order for us to be able to find ways of studying other diseases.

I have to thank the NIH leadership, starting from Francis Collins, to Dr. Koroshetz and others. A lot of these institutes really participated in this study and would not be possible unless the directors of those institutes made their resources available for the study itself. The study team consists of nearly 75 investigators, most of them put aside what they were doing and took this on top of whatever they were doing in order to contribute to the study. Anyone and everyone that I asked to help, they actually did.

A study team led by Dr. Brian Walitt, and he has done a superb job leading this entire study here. Without his help, this would not be possible.

Then we had a team of adjudicators, and they adjudicated every single patient that was on this study. That team did a phenomenal job making sure that we were studying the right individuals.

Researchers, as I mentioned, they came from all these various institutes over here, and some of them have affiliations in other universities and researchers that we collaborated with. We are grateful to all of them.

We have two panels today, an esteemed panel of study participants, and a science panel, and the panel is here as well and I'm extremely grateful to both panels and you'll hear from them as well.

I want to thank the organizers, especially Warren and Alyssa, and all the others, our press team is here, Barbara is here as well. They did a fabulous job organizing this thing. The amount of effort that went in to putting this show together today was just unbelievable. Without their help it would not be possible at all.

I want to thank the FAES for providing us coffee and snacks as well for this morning.

The goal of this study, of today's presentation, is really to discuss the findings from the NIH intramural research on post-infectious ME/CFS. We want you to hear directly from the researchers, there is no one person who understands this study in its entirety, but we have the world's experts who really contributed their time to us. I want you to hear directly from them.

Then we would like to discuss future direction, just as Dr. Koroshetz said, this is a good beginning but it's not the end.

So I want to give you a little perspective on how I got involved in this thing and I call it the tale of three pandemics. My career started with the HIV pandemic in the ‘80s. I learned a lot about what infections do to the brain through decades of research studying HIV infection. The intramural study started at the time when I was involved in the Ebola pandemic. In fact, the very early teleconferences I took when I was in Liberia for ME/CFS and that is how this started.

Then our study ended with the COVID pandemic because somewhat abruptly because we couldn't bring patients in any longer. So, really, this study was sandwiched between two pandemics, but we have learned a lot from all three of them. They have similar things. For example, with the Ebola pandemic, we realize there was a post Ebola syndrome that looks very similar to ME/CFS. With the SARS-CoV-2 pandemic, we have realized there is Long COVID, which also overlaps with ME/CFS. I think each of these infections have taught us things that we can bring to the table and try to understand these diseases in their entirety.

What is interesting is that just as I said, they are all postinfectious syndromes and overlap tremendously with one another. What is fascinating, is that with that the AIDS patients, they have persistent viral reservoirs, you never get rid of the viral reservoir, they are transferred, and the protein is being produced but they never develop any ME/CFS-like symptoms, I'm not sure why.

So just having persistent infection alone is not sufficient to explain this thing. What is interesting is, that the route of infection is quite different. If you look at post Ebola syndrome, it largely is a gastrointestinal infection, Long COVID is a respiratory infection, ME/CFS can be gastrointestinal or respiratory. The route has something to do with it or not, but there is this interesting association. These are questions that remain unanswered.

What you hear today is a description of findings related to each of these different buckets that we have put together here. In our paper, we proposed a hypothesis, it's important to say we don't really know the pathway, it is a cross-sectional study but what we have done is proposed a pathway.

So, all the researchers will discuss various aspects of this that they are experts on. I'm not going to go through this entirely because this figure it will be discussed in a lot more detail later on today.

So, the agenda for today is that you'll hear a description of the study itself. We then divided it into three major buckets, one is on immunology and omics. The second one is on neurophysiology and then bioenergetics, and lastly, you'll hear something about the data management and sharing because that in itself was a huge massive undertaking.

Then we have two panels, and we will welcome questions, there will be multiple places where there will be opportunity for discussion, and we encourage you to ask those questions.

So, this is my last slide. Here, I really wanted to directly address the ME/CFS community, and I want you to know that as physicians and researchers, we are deeply committed, and we have a very strong conviction to treating this illness and to find a cure for the disease. We are your partners, and we have the same shared goals, and I think that is important for you to realize.

As shown here, if we walk together, I think we can achieve the insurmountable. As in any partnership, healthy criticism and respect for one another will help us grow because we are really on the same side. All the same, when you doubt our intentions and pick apart every single word, you also tear us apart. It causes pain and suffering on both sides, and it demoralizes us and shatters our goals.

So, you have a phenomenal team, over 75 physicians and researchers who have devoted a huge amount of time and effort to understand the fundamental basis of ME/CFS. They are the world's experts in the field, and they took on this project on top of everything else that they were doing. When we asked them to lend their expertise to the project, each one of them said yes and they delivered.

Together, I think we can really make a huge difference in this disease, but it's important that we work together and not be against one another.

I think this is also an opportunity to discuss our findings, and I urge you to not miss the opportunity to thank these researchers when you get the chance. These researchers are really shining the light on the path that we need to follow and holding hands together, we will find treatments and cures that we desperately need.

I will stop here, and I'm going to hand it over to Dr. Brian Walitt, who's going to describe the clinical cohort and the protocol. Dr. Brian Walitt is a rheumatologist by training, and he specialized in fibromyalgia before he came to NIH and when we took on the study, he actually came and volunteered to help me put this study together. He's done an absolutely phenomenal job, he is a very astute physician and very careful. He went through records of all the patients that came through, helped identify the patients, examined them on weekends, on days, nights, whatever it took to make sure that every aspect of the study worked out really well.

Without his help, this would just not be possible. Dr. Walitt, I will let you describe the overview of the study. Thank you.

Dr. Brian Walitt: Thank you all for coming today, thank you so much for your kind words, Avi. Here we are.

I'm going to take some time right now to describe the cohort of patients that were recruited in the protocol we put together to study everybody.

As already mentioned, it was announced that we were going to study chronic fatigue syndrome, myalgic encephalomyelitis here around October 2015. Here we are, 2024, and I'm finally here to present those results to you and this is a happy moment for me to be able to show you all of this work we did.

Someone has to explain what myalgic encephalomyelitis/chronic fatigue syndrome is, and here is a slide. It's the diagnosis of clinical, we make it clinically, here is the IOM, the Institute of Medicine, criteria that says you have to have a substantial decrease in function that is over six months in time. The symptoms of post-exertional malaise and unrefreshing sleep, the cognitive complaint, orthostatic intolerance. However, that is pretty dry.

On the right is a picture that one of our participants drew to try to visualize the symptoms that she has and you can see sort of the various ways that she suffers from her ME/CFS. Muscle aches, feelings of flu, headaches, sensitivity to light, the sensation of brain fog and difficultly focusing, what it might feel like to have unrefreshing sleep, she had nausea, the idea of dizziness, and a really black hole that is the experience of post-exertional malaise.

There will be more about what post-exertional malaise is later in the presentation. We put together this protocol, when everything got started, we had to go as fast as possible. We were able to write a protocol in about a month's time to get to the IRB that did go under a fair bit of amendment over time to get it into its final form, but we did move as fast as possible to get this protocol started.

The overall hypothesis was that postinfectious ME/CFS was triggered by an infectious illness that resulted in immune mediated brain dysfunction. To do this, we wanted to conduct a cross-sectional study to deeply phenotype PI-ME/CFS participants to define its pathophysiology.

What exactly is deep phenotyping, I provided you a reference here for those looking to dive a little deeper. It's the precise and comprehensive analysis of phenotypic abnormalities in which the individual components of the phenotype are observed and described. On short, to measure everything possible, to understand all the different aspects of a person.

To do this, this is a list of most of the phenotyping measures we made. Some of these you can see are things that clinicians do, like histories and physical exams, some of these things are measurements of people's behavior, like what they eat, how they sleep, some of these things are measured to their physiology, such as their sleep, poly sonograms, and measures of autonomic testing. Remember, these things are measured of biological samples of blood, cerebral spinal fluid, and so forth.

This was an exploratory research study, most studies when people think about research are to test hypotheses. This is really to try to generate a new hypothesis to sort of give us direction to move forward in the future. For us, our mission was to explore the possible relationships between the study measurements without any prior assumptions to what those might mean.

So, participant selection was a very important piece of the puzzle here for the study. So, we wanted to make sure that everybody had a well-documented infection. This required us to look through lots of medical records to make sure that the patients had normal health prior to infection, and to demonstrate that these people who were essentially healthy developed ME/CFS following a documented infection. We felt this was important because at the end of the day if you asked us what is the cause of ME/CFS in this cohort, it's an infection. These people all had infections that cause their ME/CFS.

We brought them to campus to do detailed screening for medical and psychiatric factors, this was part of the medical record review, we did a very detailed inpatient medical and psychiatric evaluation that we will talk a little bit about in a minute. Further, we realize that in medicine, not everything that is evident at the time when you evaluate somebody, so we knew that we would have to stay in touch over the course of the study to see if things changed, if that would change how we looked at our participants. The follow-up was very important in helping us to understand the trajectory of these participants.

Lastly, we didn't want to take it all upon ourselves to decide what the proper case is. We were new in the field, as our first effort as a team to study ME/CFS. We worked with experts that were clinical experts that were well renowned in the field to help us review each of the cases, make sure they met the ME/CFS criteria and all the adjudicators had to agree that this was a good case that anyone that the adjudicator could veto and say that the case was not a good case.

We feel that our case selection process was very strong. This is just an overview of the recruitment during, while we were open, we had 484 inquiries. We ended up with 21 healthy volunteers, and 17 patients that were adjudicated to be excellent.

Why did we stop here? As Avi mentioned earlier, there is the SARS-CoV-2 pandemic that sort of shut down operations. We would have continued to recruit if we could have, however, with the uncertainty of the pandemic and the Clinical Center being closed for almost two years, we felt that to speed along the results to the community, we should shut down our recruitment and move into our analysis as fast as possible.

So, of the 484 participants that inquired, 267 of them were screened out initially. On the right side, I provide you some of the reasons why people were screened out on the telephone screening.

I personally interviewed and reviewed the records of 217 individuals, of those 146 people were screened out and you can see on the right for the reasons why they screened out. And 44 people unfortunately were stuck in limbo, that happened because of the pandemic and never got to finish the review processes.

Of the people that made it through the second set of screening, 25 healthy volunteers and 27 ME/CFS participants came to campus, and you can see here on the right, the healthy volunteers. Not everybody who thinks they are healthy are healthy. Three of the healthy volunteers were found to have medical issues that were exclusionary on their evaluation, and one healthy volunteer withdrew leaving us with 21 healthy volunteers. It's worthy to note that the volunteerism of family is always something special, and two of the healthy volunteers were actually family members of the participants.

Of our 27 ME/CFS participants that came to campus, all of them would have met ME/CFS criteria, measured that way. However, two of those participants withdrew upon the testing, four were excluded on our evaluation because one of them was found to have a very rare cancer, desmoplastic round cell carcinoma. One of them was found to have atypical myositis, another participant had parkinsonism, and a fourth had primary biliary cholangitis.

Some were also adjudicated out on review because their cases didn't really meet our high threshold for demonstrating infectious causality, which left us with 17 cases for our analysis. People always asked what infections did these individuals have, ten of the participants had upper respiratory tract infections, three were acute Epstein-Barr virus infections, one was gastroenteritis, one was atypical hepatitis, and two were related to herpes zoster infections.

The demographics are here, you'll see that we used a color coding throughout most of the presentations. We had healthy volunteers in blue, the PI-ME/CFS participants are in red shades. You can see on average, people were pretty well matched, people are essentially mid aged, in their late 30s and early 40s. We did match reasonably well on birth sex with 48 percent of the healthy volunteers being men and 41 percent of the PI-ME/CFS being men. The cohort skewed towards Caucasian, it was pretty well educated, body mass index was pretty well matched. But of course, the health of the PI-ME/CFS participants, most of them were pretty disabled by their symptoms.

All of the participants met the 2015 IOM ME/CFS criteria, 82 percent met the Fukuda criteria, 53 percent met the Canadian criteria.

So, we designed our process in three parts. The first part being a deep phenotyping visit where patients came to campus for their initial evaluation. This is where we did the bulk of these deep phenotyping measures.

Here's a sample of the calendar that a participant would get prior to coming to campus. It was a vigorous affair and a lot of our efforts as a team were to help people navigate all of the different tests and timing to ensure that they had rest to recuperate, and to sort of help cheerlead them through this gauntlet of research that we put them through.

You can sort of see in this calendar that we had to ensure for ourselves, we had to make sure there was breakfast, lunch, and time out for them to rest, we had to build that into their calendars to make sure that they can make it through the research protocol.

After they were evaluated on campus, we took all that information and we created the adjudication packets, which we sent to our expert panel. The briefing summarized their history, their physical exam, and all the medical testing we performed. I wrote narratives on each of the participants to make sure that I captured their story correctly, and every participant reviewed their story and signed off on it prior to adjudication.

The panel of experts independently reviewed each of these briefings, if they had questions, they were allowed to reach out and ask for clarifications and we would give it to them. Each of them made their independent decisions, it wasn't a groupthink situation. If they unanimously agreed, a person was invited back or was considered for case analysis. If there was a disagreement, we had the panel meet to discuss the case to see if we could get unanimity.

We would like to think the adjudicators, Lucinda Bateman, Andy Kogolnik, Anthony Komaroff, Benjamin Natelson, Daniel Peterson, and of course Avi Nath.

After that, they were brought back for a second visit to have exercise stress test performed. In this, we performed a CPET, followed it with 72 hours of measurements to try to understand the biology of post-exertional malaise. Here are some of the measurements that we put together, and a picture of a participant actually performing the CPET. Besides doing the CPET, there were measures of their bioenergetics, you see the metabolic chamber, measures of their blood, stool, saliva, even a lumbar puncture 48 hours afterwards. There were cognitive testing and even other physiological testing.

Here is an example of their schedule. Also, an impressive one. The nice thing is that there's a lot of rest after the CPET, it's really important to us with all of what our participants went through, that we try to make it possible for them to get through these procedures, and to do our most to not injure anybody.

On history and physical exam, there really wasn't too much difference between the healthy volunteers and the PI-ME/CFS participants. There were few clinically substantial findings with two patients that participated with hypermobility, and one of them had myofascial pain disorder. There wasn't too much on the neurological evaluation, one participant had a small fiber peripheral neuropathy, and one had residual weakness from their herpes zoster infection. There is one patient we thought had a migraine disorder. On polysomnography clinically, there was not a lot of difference, not a lot of pathology that we found. There was some mild sleep apnea and some mild periodic limb disorders. Any participant that we found to have sleep apnea, we gave them a six-week trial with a CPAP to see if it made a difference in their symptoms and it didn't really change anybody's symptoms in a profound way.

On brain MRI, there were some nonspecific white punctate lesions in five of the healthy volunteers and in five of the ME/CFS participants. On the clinical laboratory evaluation again, wasn't too much difference. We did a broad panel of hematology, chemistry, endocrinology, and immunology, virology, and heavy metals analysis.

On psychological evaluations, the ME/CFS participants were more often pressed and anxious than the healthy volunteers but not severely so. This sort of shows you some of those differences. In the most part, when people did have issues, they were mild. Not really that different from healthy volunteers, and I will show you that on the next slide.

Here is symptom reporting. On the left is all of the different measures that we took, it's quite of impressive list. If you look at the gray bar, what you see is that the P value for all these things are highly substantial. In general, on most symptom measures, people with PI-ME/CFS were two standard deviations, worse than what would be considered normal in the population.

On the right side, it gives you a sense of the differences between healthy volunteers and the ME/CFS participants. Again, blue and red, you can see for fatigue, they are really quite different from each other on the far end of the spectrum and that seems to be the most profound difference between the two groups. Looking at sleep disturbances, you can see that the groups are very different, again. With participants having much more symptoms in terms of the PHQ15 which is a measure of multiple systems, again, you see that the participants have many, many more symptoms than the healthy volunteers.

The MSQ attention is the measure of subjective cognition, and again, you can see that there's a difference between the groups but not profoundly so. Even less profoundly so with the right side.

With that, I would like to thank all of the team we call 3B19, all the people that directly work with me on the patients to help them through all of the difficulties of the study. Angelique Gavin, Anita Jones, Joy Kreskow, Melina Jones, Nicole Benoit, Niranjana Amin, Nicholas Grayson, Michelle Boyd, Gina Narato, Barbara Stussman, Brice Calco, Snigdha Chigurupati, Benjamin Coleman, Sean Horan, Courtney Vetter, and Ashley Williams. Thank you for your attention.

Dr. Avindra Nath: Now we are going to move into our first group, and that is on the immunology and -omics. I have a couple of introductory slides. Here we have three sessions, the first will be on immuno phenotyping. Led by Steve Jacobson, and Yoshimi. The second is on gut microbiome, and the third will be on -omics. Let's go to the next one.

Okay, so I showed you this slide previously, and so in this slide here, in this talk we will be focusing primarily on these two aspects. That is as you know, all of our patients had an infection so the question is, how does the infection affect the immune system and how does it affect the microbiome. Then, from there, how does it really affect the afferent part of the nervous system, which is how does the immune system affect the brain, as well as the changes in metabolites and the microbiome. We are going to focus on that aspect, and this slide that Brian just showed all the various testings that have been done on these patients.

And in this section of the talk, you'll hear about all of the -omics that have been done on the blood looking at the protein, the lipids, and here we listed each of the institutes that have contributed to it, the researchers who made major contributions to each one of them.

Now, the first presentation will be made by Yoshimi. Yoshimi Akahata has received a PhD from Kyoto University in Japan, and her postdoctoral training was at University of Nebraska, as well as the viral immunology section here at NINDS. She's currently a staff scientist and she works on research studies related to ME/CFS immune responses, and she studies a variety of neurological diseases including a lot of work on multiple sclerosis. She's been really interested in the immune changes in chronic viral infections, as well as inflammatory diseases. Today she will talk to you about the work she has done on ME/CFS.

Dr. Yoshimi Akahata: Thank you for the introduction, Avi, and for giving me the opportunity of the presentation. Today I would like to present our result in the phenotyping in ME/CFS using flow cytometry.

ME/CFS is a frequent and severe chronic disease impairing life quality with an underlying mechanism that is still not fully understood yet. However, immune dysregulation in ME/CFS has been frequently described including autoimmunity, CD8 T cell dysfunction, and cellular immune function, and T cell metabolism, and so on.

In addition, the disease onset of ME/CFS is often reported to be treated by infection, the link between infection and immuno-mediated diseases is well established.

Therefore, to conduct deep immuno phenotyping of PI-ME/CFS to define its pathophysiology of the PI-ME/CFS, we examined the peripheral blood, and the CSF immunophenotyping in PI-ME/CFS. In this study, we collected both blood and CSF obtained from 16 subjects with PI-ME/CFS. Also, the 19 healthy volunteers as a control to analyze these immunological markers using multiple lymphocyte measuring.

The panel included various lymphocyte markers and defined major lymphocytes, cellular subsets, activation and exertion. Overall, there was no significant group differences in percentage of major lymphocytes including: T cells, CD4 and CD8 T cells, B cells, NK cells, and monocytes. zAlso the CSF cell counts were also in the normal range. I would like to show some differences, especially two cellular subsets B cell and the CD8 T cell over the PI-ME/CFS on the next slide.

So, B cells and antibody play an important role in the control of infection in the CNS, but intracellular antibody production has been associated with both protective and pathogenic function in chronic neuroinflammatory diseases. This slide shows the development of antibody in neurological disease.

Imagine data suggests that multiple steps such as checkpoint, the defector B cell, and also defective T cell health, and also B cell activation without T cell help might mediate several neurological diseases.

In our result, the group of PI-ME/CFS shows a significant difference of B cell subset in peripheral blood including increase of naïve B cell, and also decrease of switched memory type of B cell in peripheral blood in PI- ME/CFS compared to healthy volunteers.

Unfortunately, due to the low B cell number of the CSF, we were able to analyze the CSF B cell subset, in only two subjects with ME/CFS. In those two patients, antibody secreting the B cell which contributes to B cell production – sorry, antibody production was detected in the CSF PI- ME/CFS.

So, next, we moved to the CD8 T cells, they play a crucial role in the immune surveillance and defense against the infection. After encountering antigen stimulation, the CD8 T cells activated the clonal expansion, and differentiated through the direct target cell by cytotoxic clearing. However, chronic activation and expansion by exposure of the antigen or infection, CD8 T cells are exhausted, presenting less proliferation, and less cytokine production.

In addition, this regulated the CD8 T cell and mediated the damage on to other cells in the CNS. In the CD8 T cell, increased frequency of the PD round positive CD8 T cells were detected in the CSF of the PI-ME/CFS patient. Also, the decreased frequency of CD226 positive and CD8 T cell positive were detected in peripheral blood of PI-ME/CFS compared to healthy volunteers.

PD-1 on the CD226 described as immuno checkpoint molecule to regulate T cell activation. PD-1 inhibited the T cell activation through the interruption with ligand PD-1 while the CD226 both T cell activation through interruption with the ligand CD 155. PD-1 is also reported to directly inhibit the phosphorylation of the CD226 in the intracellular domain. In chronic viral infections such as HIV, it has been reported that unspecific CD8 T cells shown increase in PD-1, and also degrees of CD226 on the CD8 T cell.

There were also significant differences of the CD8 T cell subset of PI-ME/CFS by gender. In males, the PI- ME/CFS shows an increased frequency of CXCR5 positive, CD8 T cell in the CSF. Interestingly, CXCR5 positive with CD8 T cells are reported to have a cytotoxic function against the virus infection, and also interruption with the B cells to control the antibody production in the group. In females, the PI-ME/CFS shows an increased frequency of naïve CD8 T cells, these are designed to also support gender-specific coordination of the CD8 T cell of PI-ME/CFS.

So, this is the conclusion. PI-ME/CFS has disease specific immune signatures in blood and also the CSF, including altered B cell phenotypes, elevated T cell exhaustion and activation, and also sex specific differences of the PI-ME/CFS patients. Thank you for your attention.

Dr. Avindra Nath: Thank you. Okay, next we will hear from Dr. John McCullough who is a staff scientist in the microbiome and genetics core which is part of the laboratory of integrated cancer immunology at the National Cancer Institute. He analyzes shotgun meta-genomics data pertaining to several different projects aimed at studying the role of the microbiome on human health, particularly cancer, he's interested in developing novel approaches for analyzing microbiome data and has authored a comprehensive software package on microbiome analysis. Today, he's going to talk to about the work that he has done on the gut microbiome in ME/CFS patients.

Dr. John McCullough: Hello, good morning. Today will be talking about the microbiome aspect of the study, what we did, why we did it, what we found, and what the next steps are going to be. There is more than meets the eye regarding life when we look at a human being. On all surfaces of the body, including and especially the mucosal surfaces of the gastrointestinal tract, there's a thriving community of microorganisms that have a commensal, which is meeting to say, a mutually beneficial relationship to us, which is their host.

The structure of an individual's microbiome is not static throughout life. As time goes on, this microbiome, the composition might change and is highly influenced by a panoply factors such as diet, age, geographical location, what family members you are in contact with, if you have pets or if you don't etc.

What can happen is that you can get a condition called dysbiosis, which is as opposed to ubiosis, and that occurs when there is that instability in the composition of the microbiome. That hinders or even abrogates the commensal, the mutually beneficial relationship between the microbiome and the host.

This can or cannot be reversed. The gut microbiome, if you have an unhealthy or this unstable dysbiotic gut microbiome, it has been shown to contribute to the pathogenesis of several metabolic disorders, and even mental illnesses.

How do you gauge what is in microbiome, once you have microbiome samples, how do you analyze and see what's in the microbiome of individuals? The first phase is to analyze the characteristics within each sample, so within the sample of the gut microbiome can be a stool sample, which will be reflective of the community that is currently at the time of collection of the sample in the gut microbiome of the individual who is supplying that sample.

Total DNA is extracted from fecal sample, so we are inferring what the community is based on the different kinds of DNA that we find in the sample. One of the challenges for this analysis is that the DNA sequence reads is obtained from the currently available sequences, they are in very small sizes. So, it's much like what the sequencer will give you is millions and millions of reads, which are short reads, about 100 base pairs and that represents about 0.01 percent of a bacterial genome. They are all jumbled up, so the challenge is that you have to unjumble in a valid way in order to gauge what the taxonomic community is within the microbiome sample.

Basically, there are two approaches to this. One could map the reads to known genomes, but the approach we use is a more naïve approach, was to assemble the region to larger sections called contakes. Much like you would do with a jigsaw puzzle, see which – obviously this is not done by hand, this is done by approved, true and tried software.

Then you classify taxonomically and functionally, these larger segments of the DNA, and then you quantify them. You see what proportion of each sample do these, what proportion of taxa composed the microbiome.

This was done by using a comprehensive software package which we developed ourselves and we have used in numerous high-level publications and is a tried and tested method.

The microbial species within each sample after you classify and quantify them, then the second phase of the analysis is that you have all this information within each sample, now you have to compare between samples, and how do you do that, what kinds of things are you looking for when we gauge microbiome.

Basically, two things. One is called alpha diversity, and the other is beta diversity. So alpha diversity is when you are gauging the diversity, how many different things you find in each sample. What is their share of the distribution, is it even or uneven distribution. In the example I'm giving you here, imagine four imaginary samples and I'm representing different bacterial cells of the microbial cells that these dots, different colors represent different species, so you can see that all have the same number of individual cells, but the proportions can be different.

In sample A you have 15 species with uneven distribution, so you have domination of some species at high levels and very few at lower levels. Samples B and C both have six species, but one has an even distribution so 16 percent of each species in sample B, whereas sample see, you have domination of basically two species in the whole of the sample. Species D, you have ten. When you look at beta diversity, if you gauge which two samples are most similar to each other, one could say that samples B and D, although they have different overall numbers of species, they are proportionately very similar to each other, they have the same proportion of similar shares of the same kind of the same taxa.

Given all these explanations, let’s look at the actual results from the study. When looking at diversity within each sample, these are the results that we obtained in the study. There was a statistically significant difference in the number of species, overall number of species that the CFS samples having fewer of a number of observed features as compared to the healthy controls. But when we measured the diversity with the Simpson index, which is the measure of the effective number of parties, you're weighting, giving more value to the species which represent a higher proportion of the sample, then there is no real statistical difference between the number of effective parties, number of species between the samples obtained from the healthy volunteers and those from the CFS.

This suggests that most of the difference that we see is in low-level species which are low rise of abundance. Moving on from riches to actual taxonomic composition of samples, one can get a visual representation of how similar or not the composition of two samples are to each other by algorithms that will reduce the dimensionality such as PCOA or UMAP, we did several, the one that is showing here is UMAP. You can interpret this as each dot that you can see as representing an individual sample.

The further apart that the two dots are from each other, that means that they are more different in terms of their composition. Here we see samples belonging to cases, CFS cases in red and healthy volunteers in blue. The centroids, the mean position of all samples within each group is the big dots. You can see that there is an evident separation, but that is visual.

We also did a statistical test, permutational multivariate analysis of variance, or PERMANOVA, which was applied to the sample distance matrix, and that shows that the difference in composition between the groups, between the samples contained within each group is statistically significant. In spite of there being, as expected, variation in the structure of the microbiome independent of disease status. You can see that you have some ME/CFS samples down there, which are closer to healthy volunteers samples. So on and so forth, but overall, the centroids are separated and that difference is statistically significant.

When we look at which, what other microbial species, which are either enriched or depleted, so there are about in the order of about 200 different species that we found overall in samples. If we look at which ones are increased in healthy donors, and which ones are increased

in ME/CFS samples, we find that there are about 25 different species, which are enriched in either group. By meaning enriched, I mean statistically significantly different, and at least a twofold increase. So twice as much in terms of proportion in one group rather than the other.

They all happen to be bacteria, so although we did look at viruses, fungi, arcea, and protozoa, these were all taken into account. The 25, which up or down all happen to be bacteria. Not going to go through all of them, but suffice to say that several of these species, especially those which were enriched in the CFS samples were also found to be enriched in other studies. That confirms, although it is not exactly the same species overall, this confirms the previous studies that involve the microbiome were all gram positive members of the phylum firmicutes. That is not all, that is not where it ends, in fact this is just the beginning of it, there is much more that can be done with this data.

The jams package that we used not only puts taxonomic data but also taxonomic independent with biological function, which is gauged from the predicted proteome to its function, completely independent of taxonomy. Now we are working on a second paper, which is focusing on the gut microbiome results of this cohort.

Which will correlate the microbiome, and its functions to all of these other clinical data. Also, Richard Rodriguez, a colleague of mine at NCI, he is helping us with building a TransKkingdom network which has the intent of identifying what the relationships are between these different clinical data sets and the omics. Which this may not be apparent, basically it's correlating all data to all other data and seeing how they connect to each other because you can inference the gut microbiome, fire the dietary intake and the gut the microbiome will influence the immune system or the gut brain axis, so on and so forth. We are trying to formalize and find out what the directionality is, or what the important associations are.

With this, I would like to thank everyone at NINDS, and especially at the NCI microbiome genetics core for sequencing the samples. To Richard Rodriguez, and Dr. Giorgio Trinchieri, and many in his lab for very helpful insights regarding the microbiome of this project. Especially, to Dr. Brian Walitt, to Carlotta and Jen for the ongoing undertaking of scrutinizing all these reams of data and it has been a pleasure to do so. Thank you very much.

Dr. Brian Walitt: Are we ready? I guess so. I will be talking about the various omics packages that we looked at and all the data there. What exactly do I mean by omics? Omics is the collective characterization and quantification of pools and biological molecules that translate into the structure, function, and dynamics of an organism. Simply put, trying to measure every possible thing you can out of the biofilm.

There are all different types of omics that one can measure, here are the six major categories from genomics, phenomics, metabolomics, ionomics, transcriptomics, and proteomics. For today's lecture I will be focusing on these three, transcriptomics, proteomics, and metabolomics with a dash of lipidomics in there as well. What can you do with omics? There are a number of things. First, you can identify the molecules that are different between groups. Measure the individual molecules, and you compare to see if they are different. You can then see how those differences all relate to each other by placing them into biological networks and analyzing those networks. Are those differentially expressed molecules related to each other in some way. Of course, if they are, that seems to make them more significant.

Then there is the concept of consilience, which David Goldstein told me about back at the beginning of all of this. This is this idea that you can use multiple different types of knowledge to reach new conclusions. In our omics, if we use and look at multiple different omics techniques, do those results merge into a similar conclusion across all of these different bio fluids. With that information, we hope to point the way forward for two things. One is, how can we identify new biomarkers that can differentiate persons with PI-ME/CFS from those that don't have it; and to help us identify therapeutic targets.

In this study, we looked at a lot of different omics, we looked at proteomics of about 1300 different proteins, lipidomics of about 13 lipid categories. We looked at peripheral blood mononuclear cells and looked at about 18,000 RNA transcripts. Proteomics of the cerebrospinal fluid with about 1300 proteins, and 445 metabolites. In our muscle samples, about 11,000 RNA transcripts. As you heard earlier, about our stool microbiome, about 10,000 taxa were identified.

When you have so many features but so few samples, 21 and 17, you have to use choose tools that are effective of analyzing such data. These are principal component analysis and partial least squares discriminant analysis, say that five times quickly. These really are statistics of biomarker discovery.

What are these techniques? These are linear dimensionality reduction techniques that you can use to analyze data sets where the features outnumber the samples as I said earlier. It simplifies complex data by fitting the data into lines across each of the different components while losing as little of the information as possible, this information is known as variance analyses.

Then we can use those information for each individual point, and you can see if the factors that can separate one group from the other.

So, this talks about PCA and PLS-DA. You can sort of see here, you see a set of dots that represent two groups. If you took all of the information and plotted it to a certain point, you would end up with reducing a large amount of information to a single point. Then you can see how distant or different the points are from each other. When you use a principal components analysis, a PCA, you analyze it in an unsupervised fashion which uses the data itself to try to find differences. In this case, imagine that the data looked all like Black dots to you rather than purple, green or something like that.

If you look at just the scattering of the dots, you would choose the purple arrow as the way to discriminate the data. However, the PLS-DA analysis is a supervised analysis that allows us to use the information we already know about the data, the labels of the data to choose directionality in our analysis. So, here, because we know there are differences in the groups, there is purple and green data, you would choose a different direction to analyze the data than the orange arrow.

PCA is much better for analyzing linear relationships in data, while PLS-DA is superior for analyzing clustered data and we use both in our analysis.

Here we go, we apply to PI-ME/CFS. We will start with our peripheral blood mononuclear cell RNA sequencing. So, when you look on the left, you see a bunch of different dots that really don't distinguish each other from themselves. The green CFS and the red control dots sort of overlap and there really is no separation in the groups.

However, when we analyze the data only considered ones that were different between groups, what we see is that there is some separation on the right and that this analysis suggests that there is something that is able to be separating this data and that we needed to dig a little further.

I want to thank Komudi Singh, Shahin Hassanzadeh, and Michael Sack who did most of this work. As we look at all of the data again, thinking about demographics, we separated by birth sex. Here, in PC4, you can see separated by male and female, men green, women being red, that the groups do separate. So this is suggesting to us that birth sex has an impact on the gene expression analysis and was confounding the analysis of the sample so that we might do better if we separated men from women and re-analyze the data.

This is what it looks like when you do that. On the left you can see the male data and you can see that there is a clear separation on the PCA between the eight healthy volunteers and the PI- ME/CFS men. In the middle is what happens when you do it with female data. Again, you see on enormous separation. Somehow when you reduce the number of samples, you see the separation just because of the power of sex on our analysis.

What is labeled C here is the Venn diagram, of all of the differentially expressed genes in both of the groups and in men and women only 34 percent were the same. It gives you a sense of how different the PBMCs were behaving by sex. When birth sex is considered, we were able to discriminate PI-ME/CFS from healthy volunteers.

What were these differences in men? What I'm showing you here are the data that basically tell you that this was mostly related to the STAT4-TLR9 protein-protein interactome being upregulated in men. When you dig into what that means, this is all very consistent with the expansion of the B cells and the decrease switched memory B cells noted on flow cytometry which we presented earlier. And this is consistent with that observation.

How about, what is it we see in the women? Here we see cytokine changes, lymphocyte proliferation processes in B cell interactome being upregulated. This again, is also consistent with expansion of naïve B cells and decreased switched memory B cells. So this suggests this is more than one way to get to the immunological phenotype that we presented earlier. That there may be two different immune responses that are associated with PI-ME/CFS.

So, that is an interesting pattern, but do we see it anywhere else? This is looking at our plasma proteomics, you can see here on the left our PCA, and PLS-DA analyses of all comers. This is, again, thank you to the Center for human immunology who did a lot of this work. You can sort of see that you get a little bit of separation but nothing that is really wonderful.

However, when we separated by men and women, that is a lot of separation. It seemed to be playing a role here. Unfortunately, if you look really closely, you would see the variances that are explained by these differences are very small, so this doesn't explain a lot of the differences in proteomics.

When we look at cerebrospinal fluid proteomics, on the left is all participants, then on the right are men and women. Again, you see this profound separation. Birth sex again has an impact on the proteomics on both blood and the cerebral spinal fluid but proteomics on average performed pretty poorly at discriminating PI-ME/CFS why the low variances that it explains.

How about in the muscle, here we are looking at muscle RNA sequencing. On the left again is all participants and you don't see very much separation. When we separate by birth sex, you see again a wonderful separation in both men and women, so it can discriminate when you consider birth sex. This data, thank you Iago Pinal-Fernandez, Kathy Pak, Sandra Munoz-Braceras, and Andrew Mammen to for providing this data to us.

Exactly what is different in men, here in the men you see an upregulation of the epigenetic changes, RNA processing, and fatty acid beta oxidation networks. Down regulations in Hexose Metabolism and mitochondrial processes.

In the women, you see a different set of signals with upregulation in growth hormone receptor signaling, ubiquitin transferase function and down regulation of fatty acid oxidation and mitochondrial processes.

How about lipidomics? Again, thank you to Ruin Moaddel and Luigi Ferruci for providing us this data. You see the same sort of data patterns, in all participants you see poor separation but when you separate by birth sex, you get a really nice separation in both.

How about cerebral spinal fluid metabolomics? Here you see, again, poor separation of all comers but when we apply PLS-DA to the differential expression gene, here is the first time in looking at all comers where actually you see a separation. Of all of the biofluids we tested, only the CSF Metabolomics actually separated just by diagnostic group.

It explains a fair bit of the variance, about 35 percent of the variance.

Exactly what is different in all these comers, there's mostly decreases in glutamate, dopamine, butyrate, polyamine pathways and tricarboxylic TCA pathway metabolites. These, if you follow literature, these come up again and again, not just in the ME/CFS literature, but also in the Long COVID literature.

These are differences that are decreases or parts of several pathways on the Arginine and polyamine pathway, Nucleotide metabolic pathway, and branched chain Amino acid pathway. All are sort of fundamental in cell regulation.

How about birth sex and CSF metabolomics? So when we look at men and women, when we applied the PLS-DA, again, we improve our performance at the separation. In men, this seems to be due to decreases in glutamate, women it seems to be related to Tryptophan and serotonin- signalling differences. The serotonin differences may provoke memories of this recent paper about serotonin reduction that was recently reported in Long COVID.

So, what should you take home from this? Birth sex is mechanically relevant in PI-ME/CFS. Discriminative power increases despite a decrease in sample size. This is seen with gene expression in immune cells and muscle cells, seen in proteins and blood in cerebrospinal fluid, this is seen in lipids in the blood, and seen in metabolic molecules in the cerebrospinal fluid. Everywhere we look, we found this and it seems to be profound of importance in trying to understand the nature of PI-ME/CFS.

Of all the bio fluids we tested, cerebrospinal fluid metabolomics was best at discriminating PI- ME/CFS from healthy volunteers. It was able to do so without considering birth sex, to me anyway, it suggests that the central nervous system is the place where divergent mechanisms unify into the pathogenesis of PI-ME/CFS.

Of course, these findings have important ramifications for future biomarker development.

I would like to thank everybody involved with the project, Dr. Avi Nath and Kory Johnson for their work with metabolomics. Extra shout out Komudi Singh, to whose insights into these analyses are really a person who discovered the birth sex differences in all of these different omics. A shout out to Shahin and Michael Sack, I would like to thank Iago, Kathy, Sandra, and Andrew Mammen. I would like to thank all of the members of CHI, Ruin and Luigi for all of their help in getting this analysis done. Thank you all so much.

Dr. Avindra Nath: Thank you, now I will summarize the key features from this session. First, is the immunology side. I think as you heard, the key defect that we found is really the inability of these patients to switch B cells from IgM to IgG and I think that can explain all of the poor immune response to microbial antigens.

So this will lead to, if you have persistance of microbial antigen, and I put the question mark because we generally demonstrate the presence of it, so this is a hypothesis. What it will lead to is then you have T cell exhaustion because the T cells aren't able to switch, so the T cells are trying to do their job but they really cannot do it, so they get activated and then get exhausted.

When your T cells get exhausted all you are left with is the innate immune system and that is really a bad way of trying to get rid of any kind of microbe because what it is nonspecific activation of macrophages and microglial cells that produce lots of oxidative stress and cytokines so they cause a lot of surrounding damage.

I think if there is, it can all be explained by one abnormality. Nonetheless, when we look at therapeutics you can probably target things along that chain.

The microbiome, as you heard from Dr. McCullough that while there was no smoking gun per se, there is, they didn't really find much alpha diversity but there was beta diversity. He pointed out that multiple different types of taxonomic types of bacteria were affected, one that has been talked about quite a bit is butyrate-producing bacteria being decreased. I think that is important because what we found was that in the spinal fluid also of these patients there were decreased levels of butyrate. Butyrate is not produced in the human body, the only source of butyrate is the microbiome.

The very fact that those levels are decreased is telling you that there is something wrong with the microbiome itself. So, then with the omics study, there are two things I would like to point out, one is that we are all interested in biomarkers, but the best differentiating feature that we found between ME/CFS, and the healthy volunteers was really the metabolomics in the spinal fluid so there's not a single molecule I can point at this point in time. But I think that is where your best biomarkers are going to come because it's already telling you irrespective of male, female, whatever it is, you can really separate those groups when you look at the metabolome in the spinal fluid.

I think we need to dig deeper in here, the problem is in a lot of previous studies access to spinal fluid has been very poor, but I think it is absolutely critical if you want to study a neurological disease you have to have access to the spinal fluid.

Then, we have also shown that there are multiple systems that show sex differences. I think if you're going to design various kinds of interventions, taking into consideration who you are actually treating is absolutely critical.

I'm going to end the session here and we have a few minutes for discussion so I will open up for discussion here. Any questions from the audience or from anybody else, and happy to take them. Do you have any questions online? Yes, Walter? Come on up.

Dr. Walter Koroshetz: I guess my question is kind of a general one, which is when you have a small group of controls and a small group of patients, the question is what are the differences you see and how they would stack up if you had a larger control? You have all the people in the world and you look at them, of course, that is not possible in many of these tests, but was wondering about the microbiome whether they could look at other data that they have from folks?

Dr. Avindra Nath: That is a really good question, that's true that even for the immunology here, Steve Jacobson has multiple sclerosis and all the other diseases, so we can very quickly start looking at, I think he has done some of those analyses but we didn't present it here but that is available. John, do you need to address it in regards to the microbiome? Take that microphone right there. Maybe come down here so people online can see you, that is better.

Dr. John McCullough: So, the issue especially with microbiome is that the microbiome is a moving goalpost because the microbiome isn't static. Today, I did not go to McDonald's, but I may go on Tuesday or another fast-food place that is available. These small changes, these small interventions do change my microbiome all the time.

Who have I been in contact with, did I take antibiotics, and so on. The challenge is, that especially in small cohorts, the starting point is not, you don't necessarily, there is no gold standard for comparison. Yes?

Unknown speaker: Was just going to say there are correlations. Several of these slides show that. [audio not available]

Dr. John McCullough: Absolutely, yes, right. Indeed, but one of the things that can be done is to look at all the samples together, increase sample size and also, look at multiple samples from the same patient over a long time and see because it's like in the movie, if you look at the ordination plots, the microbiome is actually jiggling about. It's more a question of how far they jiggle in the context of others.

Dr. Avindra Nath: I like the analogy, I guess that makes sense. Can you identify yourself first?

Dr. Cheryl Lohman: Sure, my name is Dr. Cheryl Lohman, I'm a patient with ME and have done research, informally on this. The cardinal symptom from the Institutes of Medicine in 2015 was post-exertional malaise. How do the results that you have just showed us fit into explaining PEM? Thank you.

Dr. Avindra Nath: I think that will be discussed further, Brian do you want to take that now or later? Why don't we take at least part of it now and then we will be discussing that in more detail later.

Dr. Brian Walitt: As we talked about sort in the design of the study, post-exertional malaise was considered in two ways. The first is that it was required as a symptom, so everybody had to have PEM. So, if you're looking at it cross-sectionally, every single person had post-exertional malaise and we measured that in four different ways. We will talk about some of that in the bioenergetics talks. Barbara Stussman, our qualitative researcher, sort of had focus groups, and we had developed a particular instrument for measuring change in PEM over time. So, you had to have it to come, the second part of the study with the CPET was designed to induce PEM while we watched. We haven't got to that part yet, that hasn't been fully studied by our team, we are still working on some of that in the ongoing directions. Really, what we did was to use the CPET to induce PEM right in front of us and then to measure that phenomenon in multiple different ways as it was unfolding.

Dr. Avindra Nath: I think it is fair to say – the pathophysiology of it and how it ties in. That is a tough question to answer because we don't quite understand the pathophysiology of PEM quite yet, with all these analyses. What we know is that it was an entry criteria so everybody had to have it which means that all these things, whatever correlates with ME/CFS is correlating with PEM. What is the pathophysiology of PEM is a question that we can't answer right now. Did you want to go? Go ahead.

Dr. Gina Montealegre: Good morning, this is Gina Montealegre, I'm a physician from NIAID and a pediatric rheumatologist. I just want to ask, first thank you. This is great, having access to all the information and the comprehensive work that you have done is something to admire an to be recognized. What are the potential expansion of these into peds? Because most of these patients are actually adults. Any thoughts about that, any future endeavors in that area?

Dr. Avindra Nath: I think that is a patient population that definitely should be studied. Conducting research on children is very hard because you know, if you want to bring them here and put them in the hospital for two weeks doing spinal taps on children is not easy, you have to anesthetize them. So, it is a massive undertaking. So we hope that what we learn here can be applied to children in some manner and maybe one could test it in a smaller cohort and not have to put them through such a huge battery of investigations, but you're absolutely right that population needs to be studied.

Dr. Gina Montealegre: Thank you.

Dr. Paul Hwang: I am Paul Hwang at the NIH, just wanted to throw out a quick question. I found Yoshimi's findings very interesting, the immune phenotyping. I guess a piece of differentiation is a problem, and in the ME/CFS population are there problems with reports of adaptive immune problems?

Dr. Avindra Nath: They are not immune deficient in some way, it's not like they are getting opportunistic infections or anything like that, if you look at the total number of these lymphocyte populations, they are equivalent with ME/CFS and healthy volunteers. They may be functional changes, but they're not to the degree that they lead to immune deficiency.

Joe Lanson: Hi, Joe Lanson, I'm a patient. You mentioned butyrate as kind of a signal for microbiome dysfunction. Is that something you would only find in CFS or is there some less of this way that could be a clinical test for microbiome dysfunction?

Dr. Avindra Nath: I'm sure you could probably measure butyrate at many different levels and any HPLC method should be able to pick it up. I'm sure people could develop it if they don't exist already. The thing is, I don't think that's going to be diagnostic of ME/CFS, because a lot of other things could potentially do it as well, it's interesting you find it here but I don't think it is going to be a diagnostic marker. We are intrigued by the fact that we could actually measure it in CFS and actually show differences, so that has never been shown before and I think that is an interesting observation. The significance of it is not entirely clear. Butyrate is very important in regulating a number of biological phenomenon, including sodium butyrate for example is used in experimentally, in various kinds of experiments, trying to modulate DNA expression and stuff like that. It actually plays a very important role, so I think it is actually quite intriguingwhat the effects might be. But I don't think one molecule alone is going to explain that disease. No.

[audio not available]

Dr. Avindra Nath: Thank you, and for those of you who are – let me repeat what he said, three important things that he mentioned. One was that the butyrate itself can affect the gut integrity, so things can then move from the gut into the bloodstream and that could have a cascade of events. The second thing he mentioned was that the innate immune response may be absolutely critical because we see stimulation of those and the third thing he mentioned was that the PEM doesn't start immediately, it actually starts much later after the exertion so the gap is a very interesting thing, something is happening during that period of time in order to lead to that and those are very important points for the discussion. Thank you very much.

Moderator: We had one question from the virtual audience. Will dietary supplementation of butyrate potentially help these patients?

Dr. Avindra Nath: John, do you want to take that one?

Dr. John McCullough: I don't know, but it might. The thing is, that butyrate production, when you have butyrate production from a microbiome standpoint, it's continually producing butyrate. So if you are supplementing it, I don't know if that will be the same. The absorption may be in a different place, you've got gut microbiome in different sections of your gastrointestinal tract, I don't really know what they are but perhaps you need butyrate being produced in a certain place. I don't know if you just give it orally if that will have the same effect or not.

Also, it could be that butyrate is not the smoking gun, it is not the absence of butyrate or not enough butyrate, that is causing all the symptoms. It's like holes in Swiss cheese, that is an added effect that may contribute to the overall appearance of the symptoms. Certainly, it's worth looking at but again, we need larger sample sizes, and we need to understand, if butyrate is being produced constantly or not, so there you go, that is what I have.

Dr. Brian Walitt: Would butyrate survive digestion?

Dr. John McCullough: It should survive digestion because it's a very small chain fatty acid and four carbons. It's four carbons and COOH, I don't see it being digested by any peptide or by any enzyme or anything.

Dr. Avindra Nath: Very well said, there are just so many different changes and I like your analogy of the Swiss cheese, plug one hole but you still have a lot of others. All right, good. Any others? If not, let's go ahead and take a small break, we have 15 minutes.

Dr. Avindra Nath: We should go ahead and get started, the second session will be on neurophysiology, and Dr. Mark Hallett will be moderating the session. Dr. Hallett is an emeritus investigator, that he just recently retired but until very recently he was at the NIH a

distinguished investigator and Chief for the human motor control section at the National Institute of Neurological Disorders and Stroke. From 1984 to 2000, he was also the Clinical director of the Institute, and he is the past president of the International Federation of Clinical Neurophysiology. Also, the past president of the Movement Disorder Society and the past editor-in-chief of Physiology. He received many awards for the work he has done, and he's really the world's expert when it comes to principles of motor control and pathophysiology of movement disorders. Dr. Hallett, thank you.

Dr. Mark Hallett: Thank you, Avi. It's a pleasure to be here to lead the presentation of the results in the study on the neurophysiology. Just to point out to be sure everyone is clear in terms of understanding any particular disease, and that is the phenotype that we are trying to understand here. One starts with etiology, what are the underlying causes of it. Those causes, which we just heard about in the last presentation somehow leads to the pathophysiology of the disorder, which we are going to be talking about, in which Dr. Walter Koroshetz was talking about also.

That pathophysiology leads directly to the phenotype, which will be of the decreased or the increase in the amount of fatigue.

We go back to this particular diagram, as you heard, we were talking about the first two columns in the first presentation. Now we are going to be talking about the next two and a half to see how that and learn about how potentially those etiological factors lead to the pathophysiology of the brain that will lead to the identification of where fatigue might arise.

These are the subset of the deep phenotyping measurements that were part of what we will be describing to you now.

This is the outline of what you'll be hearing about. Repetitive sub maximal exercise is a good way and a typical way of analyzing motor fatigue. One can understand it by looking at the elements of where the fatigue comes from, does it come from the peripheral aspect of the neuromuscular system, or does it come from the motor cortex, or the drivers of the motor cortex, which one would describe it as central fatigue. Again, Dr. Koroshetz gave an introduction to that.

You'll be hearing from Patrick Bedard, who is a neuroscientist, who's been working specifically on this project for a number of years now. Silvina Horovitz which is internationally known neuroimager who has been my colleague in the motor control section for many years.

The next topic will be central nervous system catecholamines, you'll hear from David Goldstein, in that regard. David is a senior scientist who has been at NIH for many, many years and has made many important observations on the autonomic nervous system.

We will hear more about the autonomic nervous system from Mark Levin who is a cardiologist, he is representing NHLBI in this multi-Institute project. The heart is a major target for the autonomic nervous system and it's important to understand that.

Neurocognitive issues are also important, Joseph Snow is a senior neuropsychologist at NIH with much experience and has done comprehensive neurocognitive studies that you will hear about.

Nicholas Madian is a postdoctoral fellow who was working at NIMH and is now at NINDS to continue his studies of effort in relation to the way the brain is organized. Note here again that effort is one of the drivers of the motor cortex decision-making the trade-off of reward versus effort. Again, that Dr. Walter Koroshetz introduced earlier but you'll be hearing about that later.

I'm not going to make individual introductions, the people I have already said a little bit about them we will just go through this as information, then one topic after the next. We will lead on next to or first, this is Patrick Bedard who will talk about aspects of the EMG and transcranial magnetic stimulation understanding the nature of fatigue.

Dr. Patrick Bedard: Hello, everyone. In order to make movements, the brain auto generates movements, the brain in particular motor cortex is the green strip, should there be, there we go. Seen as the green stripe, it'll generate motor commands and travel along the spinal cord, and it will activate the muscles and we will have movement. If you repeat those movements, within enough intensity it will cause fatigue.

We can ask the question as whether fatigue is represented in the brain or in the periphery, is that central or peripheral. So the goal for central fatigue is to evaluate the motor cortex excitability, or the amount of available resources to make movements. To do that, we apply

the TMS pulse which is shown there in Figure 8, a coil which is called a transcranial magnetic stimulation, over the left primary motor cortex in order to generate movements and we can measure those movements in the right thumb shown in the picture below on the right.

Here, the intensity or the amplitude of the response is an indication of how much excitability is in the primary motor cortex. What we should expect to find is that with central fatigue, we should see a reduction of the response because there is less resources for primary motor cortex to drive movements.

For the peripheral fatigue, the goal is to evaluate the muscle fatigue during fatiguing tasks. A key feature of muscle fatigue is that there is a shift in the muscle activity towards the low-frequency components. One way to measure that is with the DI index, this is the metric we will use. So we should expect that we will find with peripheral fatigue, we should see an increase in the DI index, indicating that there are still resources available to produce movements.

To induce fatigue, we designed a task in which participants had to control the green bar on the left moving it up and down by squeezing this device shown on the right. What they had to do is remain level with the red bar, which was set at 50 percent of the maximum force of a participant could do, also known as the maximum voluntary contraction. They did this test for blocks of 30 seconds, space with rest of 30 seconds. They did these blocks, many of these blocks.

The results shown first that there was no difference in the maximum voluntary contraction between the groups, here in the blue you see the controls and in the red is the ME/CFS group. During the task it became difficult for the participants of the ME/CFS group to generate enough force to maintain, to match the demands of the task. Across the box we see they cannot generate enough force whereas the controls we are able to stay level with the demands of the task.

Concerning central fatigue, the muscle response to the TMS stimulation as expected in the controls in blue, we saw a decrease of the response because the motor cortex became less excitable due to fatigue. In reverse, for the ME/CFS we saw in increase in response indicating there were still resources available to produce movement.

Concerning the peripheral fatigue or the muscle response during the task we saw that the controls remain stable or there was a slight increase, but there was a sharp decrease for the ME/CFS group indicating that there was no shift towards the low frequencies and thus there were still remaining resources in the muscles to generate movements.

So, in conclusion, we saw that the primary motor cortex, the motor network in general, remained excitable for the ME/CFS and thus had remaining resources and we saw the same thing for the muscles. This suggests that there was less driving force from the motor network.

Dr. Silvina Horovitz: Good morning, my name is Silvina Horovitz and I will be talking today about the ME/CFS field from fMRI. As Dr. Bedard told us, fatigue seems to be central, we didn't find peripheral effects. We want to understand why, and for these we want to observe how the brain areas engage or disengage during the process of fatigue as it's happening.

For that, what is functional MRI? It is a technique that we use with a scanner as you can use with any of the other magnetic imaging studies but has the ability to follow neural activity. The neurons are activated, it will need supplies, the supplies come through the oxygen, the blood will become oxygenated and the scanner is magnetic, the properties of the blood, oxygenated and deoxygenated are different. Therefore, the scanner can have alterations, and that will become the signal as you can see in the figure on the far left.

That is a map of the areas that participated in the task. Sorry, the other way.

For these fMRI, we don't have an absolute measure, it’s not that the area and the level of signal will tell exactly a number, but rather the difference between two different conditions. In the block design, as shown on the top, you have rest and task or an activity and that is what we are comparing. What is important to note is that because it's a relative difference between the two conditions, the value that we are seeing is again, could be a change in activation or a change in the condition that we call rest.

So, for our study, we use the same paradigm, just described by Dr. Bedard where we use the rest as relaxing the hand and the task was the grip force. Using the monitor used before, and also asking the subjects to maintain that red bar that will be at 50 percent of the maximum contraction that they could do.

The two questions that we wanted to answer now within the brain is whether the deficits come from the motor network, or the changes in fatigue come somewhere else where what's happening in the whole brain because the motor cortex is the motor network is our obvious place to look in the motor task, but the brain acts as a network and more integrated.

The results that we have are similar, again, the tests to what is find in the experiment with TMS and EEG. The signal, the number of blocks that's controlled is higher than the number of blocks the patients were in the ME/CFS, basically we were able to perform, however, everybody did the task and when we look at the motor task activation, we then see that both have activity in the motor cortex and the supplementary motor cortex. Indeed, we don't see in that network a significant difference when we compared the signals.

When we look at the whole brain analysis, we do find differences. Again, here we need to do the analysis slightly different, so for these parts of the analysis, we divided the 16 blocks that the subjects did in four chunks. So each, when 2-way ANOVA where we compared how the signal evolved over time and across groups. What we found is the difference of these area that is a temporal-parietal junction and some areas in the parietal lobe. These areas are related with sensorimotor integration, including motor control.

What we find is that at the beginning of activation, at the beginning of the trial the first four blocks, there was no group difference in the way that these areas were engaged. As we progress in the blocks, we can see the difference in behavior, we can also observe in the blue line that the signal increased up to the point of fatigue and then goes down. However, in the ME/CFS group the signal continuously shows a decrease.

In summary, we can say that the motor network engagement shows a similar pattern for both groups. This suggests that it is not the cause of fatigue. When we look at the whole brain level, the areas that show differences in the temporal-parietal junction on the temporal lobe where the areas of high order integration show the difference, and these could indicate some decreased motor control signaling or some changes in the sensorimotor matching.

Dr. David Goldstein: I am Dave Goldstein, I direct the autonomic medicine section in the clinical neurosciences program. Today I will be talking about the autonomic and catecholaminergic findings in the ME/CFS group compared to healthy volunteers.

It's important that the outset to understand that the automatic nervous system isn't one thing. Langley defined the autonomic nervous system in terms of enteric, parasympathetic nervous system, a phrase that he invented, and sympathetic nervous system. Subsequently it was found that there are specific chemical messengers associated with these different components of the autonomic nervous system. In particular, acetylcholine is not only the chemical messenger of the parasympathetic nervous system but also is the chemical messenger of the sympathetic system which mediates sweating. Or is one of the main determinants of sweating.

Although, Walter Cannon defined the sympathoadrenal system as a monolithic emergency system to maintain homeostasis, a word that he coined in emergencies. Actually, these are different components and are regulated differently as I will get into in a little bit.

The chemical messenger of the sympathetic nervous system in cardiovascular regulation is Norepinephrine. Epinephrine or is synonymous with adrenaline, is the main effector of the sympathetic adrenergic system and the endocrine system.

One of the key autonomic function tests that is done is the tilt table testing. This is because when a person is tilted up, there is a decrease in venous return to the heart and you're examining the ability to counter that decrease in venous return by a variety of compensatory homeostatic processes.

This shows the arrangement at the time of the autonomic function testing that started with the Valsalva maneuver but I will not be going into any of the results of that so I will be focusing on what happened during head up tilt table testing for up to 40 minutes.

Here are the physiological data or some of the physiological data. As the convention for other talks, healthy volunteers are in blue and the ME/CFS group is in red. You can see that the heart rate increased in both groups, increased identically. Finger systolic blood pressure went up in both groups, kind of an interesting phenomenon, you would think that the blood pressure should not go up with tilting, but that is what was found.

Stroke volume went down in both groups, this is stroke volume estimated by applying an algorithm called beat scope to the finger cuff with a blood pressure signal. Skin electrical conductance which is a measure of sweating the sympathetic cholinergic function went up in both

groups. There is a suggestion of an augmented decrease in stroke volume in the chronic fatigue patients.

In terms catecholamines you can see that the levels of norepinephrine went up and stayed up during the tilting, but the groups did not differ. On the other hand, when it comes to Epinephrine, there was a progressive increase in Epinephrine and the groups tended to differ. You can see based on the individual results here that some of the ME/CFS patients had large, Epinephrine responses and that explains the increase in the mean values, but there's a lot of variability.

To summarize the tilt findings, there was no orthostatic hypotension based on systolic blood pressure by the finger cuff method in either group. There was no increase in the frequency of postural tachycardia syndrome defined entirely by the heart rate response to tilting. The difference that you see is not statistically significant.

The groups did not differ in the frequency of tilt-evoked sudden hypotension, which is also called neurally mediated hypotension although it is probably not neurally mediated. There is no difference in the frequency of tilt evoked symptoms such as pre-syncope, nausea, that sort of thing.

When it comes to CSF, this is a schema that shows the sources of the different levels of catechols in the spinal fluid. Importantly, you can see that Norepinephrine is stored in vesicles based on tyrosine hydroxylase converting tyrosine to dopa, dopa decarboxylase or L-aromatic-amino-acid decarboxylase converting dopa to dopamine. Dopamine gets taken up into the vesicles and is converted to Norepinephrine.

The main measure of Norepinephrine stores is not Norepinephrine itself, it is DH PG, the main neuronal metabolite of Norepinephrine. The main index of dopamine stores isn't dopamine, it's DOPAC, dihydroxyphenylacetic acid, which is the main neuronal metabolite of dopamine.

This is what we found, you'll notice that dopa, the precursor of the catecholamines is significantly decreased in the ME/CFS group compared to healthy volunteers. Most notably, DH PG, the main neuronal of the metabolite of Norepinephrine is quite decreased in the ME/CFS group.

We did not find evidence for orthostatic hypotension in PI-ME/CFS, there were nonsignificant increases in postural tachycardia syndrome defined entirely by heart rate. There was no difference between the groups in the tilt-evoked sudden hypotension. There was possibly augmented vasoconstriction or fall in cardiac venous return during tilt in the ME/CFS group.

Plasma Norepinephrine was normal during supine rest and increased normally whereas there was a suggestion that some of the ME/CFS groups had large Epinephrine responses to tilt, but the overall impression of sympathoadrenal imbalance which is to say larger increase in sympathetic adrenergic activity than sympathetic noradrenergic. Finally, the CSF catechol abnormalities in PI-ME/CFS suggest decreased central catecholamine biosynthesis resulting in decreased Norepinephrine stores. Thank you.

Dr. Mark Levin: Hi, I am Mark Levin, a cardiologist in NHLBI as Dr. Hallett mentioned. I study arrhythmia in sudden cardiac death and an element of that has to do with studying the autonomic nervous system which is what heart rate variability does and what I will talk about today.

The heart is a pump that is coordinated by the cardiac conduction system, and the system is pictured here in this cartoon. Normal beats conducted properly which means those beats that are initiated in the sinus node and progress to the AV node throughout the rest of the heart are considered normal beats. The electrical activity that proceeds through the heart can be graphed like the picture here, and one can name components or complexes in that graph, so there is the P wave, there is the QRS complex. When we talk about the distance between the two QRS complexes, we talk about the RR interval. When those beats are normal beats, meaning properly conducted through the transduction system, we can refer to them as the NN interval which is the abbreviation that we will see in the rest of the talk here.

So, HRV involves comparing normal beats mathematically to quantify autonomic nervous system activity. This analysis assumes time differences between different normal beats that are attributable to differences in autonomic nervous system activity. That activity can be further broken down into sympathetic and parasympathetic activity. Those elements can be quantified with heart rate variability, parasympathetic measures are pretty well agreed upon. It is pretty well agreed that the sympathetic measures are not great.

So, some of the different types of mathematical approaches you can take are pictured here. Those include the time domain, which involve straightforward standard deviation of the NN intervals. Frequency domain, which assumes that a waveform can be decomposed into a

composite sine wave as shown here using what is called the FFT transformation and those composite sine waves can be further quantified and compared using the spectral power analysis as shown here.

Finally, there is what is called nonlinear approaches, which are higher order mathematical analyses, which assume no predictable proportionate relationship. One of those types of analyses are pictured here, which is called the Poincare plot. That simply involves graphing in the RR interval as a function of the subsequent RR interval. I will talk about one measure, which involves this perpendicular axis that is called the SD1 interval.

We did our analysis based on the consensus guidelines. You can see the references for those guidelines here. In doing these analyses, we chose the heart rate as the surrogate of the sympathetic activity, while there was no statistically significant, we found a trend towards significance in that their heart rates in ME/CFS subjects looked like it might be greater than that of controls.

With respect to parasympathetic activity, we used several different timed domain markers including rMSSD, and pNN50. We found ME/CFS subjects had diminished values in both of these parameters.

Similarly with respect to frequency domain analyses, we found high frequency power diminished in ME/CFS subjects compared to controls. Similarly, we found the same thing in SD1 as well, diminished in subjects compared to controls. We found that there are composite measures that measure overall, heart rate variability, those include SDNN index and SD1, we found both of those diminished in subjects compared to controls as well.

In conclusion, heart rate variability assessed from 24-hour ambulatory ECG suggest that ME/CFS subjects have decreased parasympathetic activity as shown by pNN50, high-frequency power and SD1. There is a suggestion that increased heart rate in ME/CFS subjects compared to controls might suggest increased sympathetic activity.

Finally, ME/CFS subjects have decreased overall heart rate variability as compared to controls with respect to SDNNi and SD1. Thank you very much.

Dr. Joseph Snow: Hello, everybody. My name is Joseph Snow, I'm an intramural clinical neuropsychologist in NIMH. So, we performed cognitive testing in ME/CFS patients because of their complaints of cognitive difficulties or brain fog, which has been described as a core symptom in ME/CFS. In addition, it's been asserted that cognitive exertion triggers post-exertional malaise. Finally, some prior studies have found cognitive deficits in ME/CFS.

The goals of our neuropsychological evaluation were to use standard psychometric methods to evaluate cognitive functioning in ME/CFS and evaluate the subjective complaints of the ME/CFS volunteers.

So, a valid neurocognitive evaluation has several requirements. There needs to be standard administration, that is, an examiner must follow the same rules for all respondents. There must be optimal task engagement, there needs to be objective scoring, all trained evaluators yield the same results within a margin of error and normative comparison. Testees are compared to others that are similar to them such as in terms of their age and education.

As part of our battery, we administered a variety of different types of measures. So-called subjective measures such as asking testees to test to rate their own symptoms of depression, symptoms of anxiety, or their cognitive symptoms or their own level of task engagement.

We also included performance validity tests which are quantitative measures to determine if there is an acceptable level of task engagement, and thereby assist in determining if the evaluation itself was valid.

Here are some sample neurocognitive tests. On the left, we have a list learning task where a list of words is read to the volunteer several times and a number of words are recalled in each trial is recorded as well as following the delay.

In the middle panel we have a design learning task. Several trials are presented and the volunteer has to recall the designs after each presentation and following a delay.

On the right is a card sorting task that is used to assess executive functioning.

We did a whole bunch of tests, some additional tests would be asking volunteers to generate words to cue or asking them to rapidly place pegs in slider holes or to do other tasks like mental arithmetic or coding tasks.

So, the data is collected such as the number of errors, and the raw scores, time to completion. And then we also compute through the normative process where raw scores are converted to scale scores, T-scores are converted to standard scores, each has its own means and standard deviation.

So to summarize, we collect several types of data including subjective measures, performance validity, and performance-based measures that is, the neurocognitive data proper. So, once– one subjective measure is the MASQ that is the multiple ability self-report questionnaire, and on this, for this questionnaire, we asked volunteers of the ME/CFS participants and controls, questions about their current language, attention, verbal memory, visual memory, visual perception etc.

We found individuals with ME/CFS in the red describe more problems in each of these categories than controls. Then we also measured depression, this was mentioned earlier, and we found that ME/CFS volunteers described more symptoms of depression and anxiety than controls.

Although they are not very elevated. Either of them.

We also used something called visual analog scales to have our participants self-rate their mental fatigue and physical fatigue, their engagement, and the task, and fatigue appears to go up across time for everybody. The ME/CFS volunteers have more self-reported mental and physical fatigue prior to and during testing so we did multiple measures across time. Both the ME/CFS and control groups endorsed self-reported fatigue at an equal rate across the testing session, so we didn't see a divergence of the two lines.

In terms of performance validity tests, which are designed to ensure adequate task engagement, there were no differences between ME/CFS volunteers and healthy volunteers. So, individuals with ME/CFS do not appear to be unengaged in doing well on the neurocognitive test.

In terms of performance on standardized neurocognitive measures, and these are the measures that have been well validated and been used for decades, we found that ME/CFS volunteers performed in the average range, and that the yellow line attempts to depict that for each of the different categories of tests.

The performance of the ME/CFS according to our statistical methods did not differ from our controlled volunteers.

So, to summarize, ME/CFS volunteers have a satisfactory level of task engagement. ME/CFS volunteers do not have invalid task performance. ME/CFS volunteers have more self-reported deficits in attention, verbal memory, visual spatial, language and visual memory. ME/CFS volunteers also have significantly more self-reported depression and anxiety symptoms. ME/CFS volunteers have more self-reported mental and physical fatigue prior to and during testing battery but both ME/CFS and control groups endorsed self-reported fatigue at an equal rate across the testing sessions. Despite those findings, ME/CFS volunteers performed normally on standardized measures of cognition and their performance cannot be distinguished from that of controls. Thank you.

Dr. Nicholas Madian: Hello, my name is Nick Madian and I'm going to be talking about our findings related to effort. Specifically, I will talk about how effort can be thought of as a feeling and not just as an action. Why we feel effort, effort discounting, effort preference, the effort expenditure for rewards task or EEfRT, how the EEfRT is interpreted, our findings, similar findings in other neurological disorders and conclusions and directions for future research.

When people talk about effort in their daily lives, they often use it to describe action, something that is under conscious control. Merriam-Webster's first entry for the word effort calls it a conscious exertion of power, however research on effort suggest this common definition may not be entirely accurate. We now know that effort can be thought of not only as an action, but also as a feeling that we get when we take certain actions. Starting in the early 1900s, researchers noticed there was something inherently difficult and disagreeable about performing certain types of work. If we think about it, we realize this feeling is everywhere in our daily lives, we feel it every time we walk up a flight of stairs, or carry something heavy, or bend over to pick something up. It's this feeling that we are calling the feeling of

effort or the sense of effort. We can identify that this feeling is unpleasant and aversive and that it gets stronger and more unpleasant the more energy the work requires.

We also know that the exertion of effort is not entirely or maybe even mostly conscious despite what Miriam Webster says. When the feeling of effort gets to be too much, your brain will automatically back off whether you otherwise want to keep going or not. We have also learned that the feeling of effort can change based on context, even as the effort exerted stays the same. When spending energy allows one to obtain something good or avoid something bad, the feeling of effort can be reduced, and we can exert more of it.

We also know that the feeling of effort can change based on fatigue, the more fatigued we are, the more unpleasant effort tends to be. To generate feelings of effort that can change based on context, the brain must have systems that account for both the energy being spent as well as the rewards to be gained and the punishments to be avoided for spending that energy. Furthermore, the systems must be able to compute some sort of ratio of cost to benefits, the results of which influence how the effort feels. We can think of this as being a bit like the system trying to figure out which side of the scale is heavier, or whether the solution to an equation is positive or negative.

The process by which the brain figures out this cost-benefit ratio is called effort discounting.

There's one more layer of complication to this process. The weights that are given to the energy costs, obtained rewards, and avoided punishment seem to differ on a person-to-person or brain to brain basis. For example, to the exact same energy cost and the exact same context, two brains can assign two very different weights, which means that the feeling of effort can be different for different people.

The same brain might even assign different weights to the same cost at different points in time. For example, when one is starting a task versus when one is fatigued. In fact, this change in waiting may be an important part of the inexperience of fatigue itself.

A lot of this process is thought to occur within a cluster of brain regions called the valuation network. Another region of the brain that may be important to this process is the locus coeruleus, a tiny region in the brainstem which produces the neurotransmitter norepinephrine for the rest of the brain. The locus coeruleus has also been implicated in both the experience and exertion of effort and is connected to several brain regions in the brain valuation network. importantly, the functioning of these brain regions does not appear to be under conscious control, the weights of rewards and costs are not something people choose, rather it seems that the valuation network may simply be wired differently from person-to-person resulting in different people experiencing the feeling of effort in different ways.

The interindividual variability throughout this process is called effort preference. Some variability in effort preference is normal, for example, we can consider hypothetical people Aand B who are asked to do the same exercise, 20 push-ups for the same reward of $20. Person A’s valuation network might assign large weights to energy costs, and small weights to reward benefits. We believe this person would feel effort from the push-ups strongly and all other things being equal would be less likely to accept the offer. In contrast we also have person B who’s valuation network might assign smaller weights to energy cost and larger weights to reward benefits which leads to them feeling less effort and makes them more willing to accept the offer.

Although different, both are examples of healthy valuation network functioning. But sometimes the network does not function properly, it appears that certain neurological diseases can disrupt or even damage the valuation network affecting the effort discounting process and radically changing the way that effort is experienced. Diseases like Parkinson's or frontotemporal dementia, certain types of strokes and certain types of brain damage have been found to damage valuation network brain regions affecting effort discounting, effort preference and the feeling of effort itself.

We wondered whether something similar might happen to people with ME/CFS. To test whether there were any behavioral signs of disrupted effort discounting in people with ME/CFS, we administered the effort expenditure for rewards task. This is a well validated task that is frequently used to quantify effort discounting and fatigue ability. It and similar tasks have been used to study effort in several neurological disorders. It requires participants to perform a series of trials consisting of button pressing subtasks with two difficulty levels. Participants selected the difficulty levels themselves based on the reward at stake which varies from trial to trial.

Each trial begins with the five second choice period in which participants are presented with information regarding the reward magnitude of the hard subtask for that trial and the probability of receiving any reward for that trial. After choosing the hard or easy subtask, participants

make rapid button presses to complete the chosen subtask, they then receive feedback on whether they successfully completed the task and are then told whether they received any reward based on the reward probability and whether they completed the task.

Altogether, the task runs for 15 minutes. The EEfRT and similar tests generate patterns of responses which can be interpreted as follows. The leftmost graph depicts how two hypothetical people represented by the purple and teal lines will respond to the task as reward value increases. Both people assign equal weights to energy cost initially and start at the same point on the graph. The person in purple weighs rewards substantially and will engage with hard tasks more often when more reward is offered. In contrast the person in the teal assigned little to no weight to reward so no amount of rewards is enough to get them to increase their engagement with the hard task. The middle graph depicts a baseline difference in effort discounting. The person in purple weighs energy cost less heavily than the person in teal, thus they demonstrate a stable tendency to engage with the hard task more often their lines start at different points.

In the rightmost graph, both people assigned equal weights to energy cost initially but only the person in teal gets fatigue, the person purple is immune to fatigue, and the weights do not change at all throughout the task.

We found that for the same levels of incentives, people with ME/CFS were significantly less likely to engage in the difficult tasks as compared to healthy controls. This indicates that something about the effort discounting process is altered or disrupted in people with ME/CFS. On this graph, this is indicated by the significant gap at baseline between the blue line for the healthy controls, and the redline for the people with ME/CFS. However, we did not see an indication that the reward weighting was different between groups, both groups engage in the hard task more as rewards went up.

We also investigated whether the people with ME/CFS effort preference changed in any different rate compared to the healthy controls. In essence we wanted to see whether they fatigued faster. We did not see an indication of this in our data, the probability of engaging with the hard task declines at approximately the same rate for both groups. Although we did again see a difference at baseline which persisted throughout the task indicating differences in effort discounting.

Importantly, we also found a strong positive correlation between levels of the neurotransmitter norepinephrine and the proportion of hard trials chosen in people with ME/CFS. This suggests a neurobiological underpinning potentially involving the locus coeruleus valuation network for disrupted effort discounting in people with ME/CFS.

Notably, effort has also been studied in Parkinson's disease and findings look remarkably similar to our findings in people with ME/CFS. In a 2015 study, Chong et al demonstrated people with Parkinson's disease did not engage with the physical task as readily as healthy controls for the same levels of incentives. Importantly, this was a task that the people with Parkinson's disease and the healthy volunteers could perform equally well so that the group difference was not related to the motor dysfunction associated with the disease.

Interestingly, once given medications that increase levels of the neurotransmitter dopamine in their brains, the people with Parkinson's disease began to engage with the physical task much more readily than the healthy controls for the same levels of incentives. Increasing dopamine in the brain changed the effort discounting process, underscoring that effort discounting stems from neurobiology and is not a conscious choice.

To conclude, it appears that people with ME/CFS experience some alteration or disruption of effort discounting which leads to altered effort preference, this appears to be related to the levels of the neurotransmitter norepinephrine. Although more research is necessary it may be the case that these alterations in effort discounting are related to the post-exertional malaise often experienced by people with ME/CFS.

Further research on valuation network damage or dysfunction as a possible contributor to the symptoms is also warranted. A closer investigation of valuation network functioning in people with ME/CFS is already underway.

Dr. Mark Hallett: So, I hope that you understood all of those things that were presented by the team. They did a very good job, I think, in giving you these important data. Now my job is to try to synthesize it a little bit better, perhaps for you so you can understand how we think about it at the present time.

So, the first point which I think is fairly clear, is that there is fatigue of continuing muscle contractions. This is a relatively obvious point, patients have indeed muscular fatigue. The question is, where does this fatigue of motor performance come from. As we were talking about, does it come from the central nervous system, or does it come from the peripheral nervous system, and we have been hearing about the central origin, but we wanted to prove it here in this particular study.

There is some disuse atrophy in the patients but the critical finding in our study is that there was no increase in the slope of the Dimitrov index, which comes about if there is fatigue in the muscle.

Let me discuss briefly, energy metabolism in the muscle. In order to produce ATP, which is energy, you need to have a substrate such as glucose, and you can then metabolize that potentially with oxygen. You can create through the process of glycolysis pyruvate, which is the substrate utilized, and if you have oxygen, preferentially you have aerobic metabolism, which will produce a lot of ATP.

People prefer to use that pathway as long as they can. If you run out of the ability to use oxygen, because you are fatiguing the muscle, then you will switch to an anaerobic mechanism in which you produce lactate. Lactate is what is the driver of the Dimitrov index. If you are not at the stage of changing the Dimitrov index, you're not shifting to anaerobic metabolism, you are still performing aerobically, and that is the source of the statement that Dr. Bedard made when he said there are still resources available to the patient as far as the muscle is concerned.

So, it doesn't appear to be due to a problem in the muscle, it is not due to peripheral, and then the question was, is it due to a problem in the motor cortex, which is the direct driver from the central nervous system to the muscle. You heard that there was no abnormality in the motor evoked potentials, which is a measure of the driving from the motor cortex to the muscle.

There was no evidence that the motor cortex itself was problematic. Actually, you saw in the newer imaging results that Dr. Horovitz presented, that when the motor cortex itself, the supplementary motor area were compared, there was no difference between the groups. There is no evidence that the motor cortex or the direct drivers of movement are in fact abnormal.

Now, this is again a simple diagram of the way that the system is organized, we have the muscle, and the primary driver is coming from the primary motor cortex and some associated regions such as the supplementary motor area. The motor cortex gets driven by various factors through the rest of the brain, that is why the motor cortex does what it does, it has to be, to get these driving factors to actually decide to make these different movements.

There appears to be then a problem with central fatigue not arising from the motor cortex, but arising from the drivers of this.

I think in trying to understand that, that is where we come to a very interesting correlation in relation to the deficiency of catecholamines. You heard that norepinephrine has come up with a couple of times as being significantly affected, you've also heard a little bit about serotonin earlier on as being a potential problem. There are a number of these catecholamines in the brain, we see this manifestation in deficient autonomic control of the blood pressure of the heart, but it may also affect brain metabolism directly and I think that these findings in the autonomic nervous system give us a marker to a certain extent of what is going on in the central nervous system.

We have all of these neurotransmitters, cholinergic system, serotonergic system, histaminergic system, dopaminergic systems, noradrenergic system, all in the brainstem and they innervate the entire brain. We have influences of all these neurotransmitters everywhere, this is a blowup of the brainstem region. You already heard about the noradrenergic source in the locus coeruleus giving rise to innervation of different parts of the brain.

Now, energy is also needed to function in the brain, it's not only in the muscle. Energy metabolism is a little bit different in the brain as it is in the muscle. Generally, we have oxygen most of the time but still, it's important to have a substrate such as glucose, and you have to produce pyruvate. It turns out that neurons like to have lactate when they want to fire quickly. Lactate is an important substrate in the brain coming from glucose in the long run, but in the short run you can just have lactate itself.

Lactate in the brain is supported by the glial cells, the astrocytes in the brain supplied neurons with a lot of the lactate that they use when they need it. One of the other interesting features that turns out, is that norepinephrine is an important neurotransmitter that helps the

astrocytes transmit lactate to the neurons. We see that there is an abnormality of norepinephrine that is present there, norepinephrine is helping the astrocytes supply norepinephrine to the neurons, therefore, there could well be an energy problem with this function within the brain.

So, we have all of these drivers and what we see is that there is a specific driver that is diminished in the brain that we have when we look at the whole brain imaging, you heard from Dr. Horovitz she said the decline in the activity to parietal junction and nearby parietal lobe. These regions, as she noted are important for sensorimotor integration, both the sense of feeling about movement, the interpretation of movement, and also the driving of movement. That is a part of their role, but they can also play a role presumably in cognition and other aspects as well.

So, there is this decline, there appears to be a decline of energy for that effort that I pointed out and it's presumably what is experienced as central fatigue.

So, what we understand then is we heard about the etiological factors here, these abnormalities in the etiological system that we have, which will then potentially influence the hypothalamus and brainstem to reduce the neurotransmitters. They then influence other parts of the brain, and that will eventually give rise to the different symptoms that we have been talking about, and we have emphasized here, particularly decreased motor control.

It is also the case, of course, that these systems will drive the cardiorespiratory system, and you'll be hearing more about that in the next series, but for the moment, we will stop here with this particular part of the pathophysiology.

That is the end of our presentation, and we will be happy to deal with questions. Perhaps the panelists who made presentations can come down so that we can answer the questions if anyone might have for us. Thank you.

A question?

Dr. Cheryl Lohman: Yes, my name is Dr. Lohman, and I'm a patient and also have done informal research. I would like to direct this question to Dr. Snow. When you studied the subjects, for instance, when you were doing the neurocognitive studies, did you also correlate that to whether the patients were symptomatic with their brain fog at the time? We know fog comes and goes, right? I'm wondering if you looked at that and also, the same with muscles. Did you look at the findings of the muscles when the patients were experiencing their PEM. Thank you.

Dr. Mark Hallett: Good question, the answer should be given at the microphone so people online can hear. Maybe we can come to this microphone.

Dr. Joseph Snow: Yes, correlation, as a statistical term, no. But as he can see from the visual, I presented the visual analyzer scales and people did endorse during our session that they were fatigued, and that they were having problems both physical and mental fatigue at the time. So that would indicate that there was some and that it went up as the session progressed. I wouldn't say that we were able to necessarily capture them while they were having brain fog, like we didn't, I'm not sure whether, Brian did you want to say something?

Dr. Avindra Nath: There may be another way of addressing that question. You showed that the subjective complaints were quite significant, so in that population of patients that you saw, how many of them were complaining that they had complaints of neurocognitive dysfunction?

Dr. Joseph Snow: All of them.

Dr. Avindra Nath: 100 percent, that is basically the answer is 100 percent of them have brain fog.

Dr. Joseph Snow: Yes.

Dr. Mark Hallett: I have a similar question about the muscle, at that stage of being tested, where they at sort of the baseline, or did you feel that they were already complaining of muscle fatigue? Muscle fatigue was a symptom that they had ordinarily.

Dr. Patrick Bedard: At the beginning of the measurements, we considered, both groups were at the same, they were at the same level. As they did the test and in time their responses completely changed.

Unknown speaker: Are we talking over a day or two days?

Dr. Patrick Bedard: That wasn't part of – we didn't do that measurement.

Dr. Brian Walitt: Hi there again. Over the course of all of these tasks, we were performing visual analytic skills. The functional MRI, the beginning and the middle and the end we were present progression of their symptoms and understanding the changing of the task and whether they were distorted. In general, they were starting with sort of a high fatigue or high symptomology all the way through. When we had the participants tell you about their experiences, many of them said that they were essentially PEM for most of the evaluation when you ask them directly.

We also measured questionnaires throughout the week, and essentially throughout the week they always had really high levels of both physical and cognitive symptoms.

Dr. Mark Hallett: Great question. Thank you.

Dr. Paul Hwang: Paul Hwang, NIH, this is a question for Patrick and maybe others as well, so this motor cortex M1, I don't know much about this TMS stimulation but is there a possibility that it could be fatigue of the brain secondary to medical grade disorder? I'm wondering whether in primary mitochondrial diseases, where there similar findings observed when you did this kind of testing, or TMS mostly?

Dr. Mark Hallett: Right, motor cortex Metabolism was normal at baseline, there wasn't any change in the way that the motor cortex was functioning and as you saw with the imaging, it functioned normally in terms of going up with the exercise. So the motor cortex metabolism was normal, and changed normally with exercise. There was no evidence of that abnormality of the cortex. The abnormality was, as you see there, was in in a different part of the brain. That, as I was pointing out could be due to a metabolic abnormality.

Dr. Paul Hwang: I guess I'm just trying to put it all together like with David's finding of the dopamine metabolism – I guess catechol metabolism, those are required, the mitochondria. So with the dysfunction, you could kind of put all of this together in the brain.

Dr. Mark Hallett: Yes, that is sort of the way we were doing it, yes.

Unknown speaker: Thank you, I have two questions. One is, was there a correlation between anxiety and the levels of catecholamines. The second question is, if there wasn't any reference to nucleus accumbens when you are talking about reward and wonder whether or not that is something that you just couldn't see, or the reason why there were no abnormalities. Thank you.

Dr. Mark Hallett: In terms of the imaging, there were no abnormalities in the nucleus accumbens, that did not show any difference between those. The other question about correlation between anxiety, these are, we did many different correlations across different things. I don't recall whether we did that particular correlation or not. Brian, do you recall whether that correlation was done?

Unknown speaker: I think that epinephrine would be worth looking at.

Dr. Mark Hallett: That is correct, we didn't do it. Okay, if we did it, we don't recall that we did it. Anyway, but that would be easy enough to check.

Moderator: Dr. Hallett, we have questions from our virtual audience. The creators of the EEfRT state that in their original validation article that the EEfRT data is not valid if participants are not able to consistently complete the task, why did you proceed with interpreting the PHTC data as evidence of impaired effort preference when the data appears to be invalid according to the guidelines set by the task readers?

Dr. Nicholas Madian: So, I wanted to field this one and express my thanks for this question and the opportunity to provide this clarification. I was informed that this question has been raised and I wanted to field it because I've done a lot of work with this task and similar tasks. I'm very familiar with the original publication on the EEfRT that the question mentioned, and nevertheless, I still really want to

make sure that I did my due diligence, so I spent a significant amount of time before this meeting reviewing the publication in question especially closely to make sure that I'm representing it accurately.

What the paper describes, is that the EEfRT was designed so that the sample of patients used within that original study could consistently complete the task. This does not mean that everyone who takes the task must be able to complete the task without issue, for the administration or data to be value– valid or interpretable.

It seems that the creators wanted to ensure that in general, as many people as possible would be able to complete the task, but without compromising the testability to challenge participants. Furthermore, I think it bears mentioning that although ME/CFS participants did not complete the task with the same 96 to 100 percent rate as the participants in the original study, or at the same rate as our healthy controls, they still completed the task a large majority of the time.

To wrap things up to answer the question, consistently completing the task is not a requirement for a valid EEfRT test administration and by all accounts, we believe our data is valid and is thus interpret both as a measure of impaired effort discounting.

Moderator: Thank you, Nick. Then we had another question, what is the rationale for reframing the EEfRT as a measure of preference rather than to using an established interpretation?

Dr. Mark Hallett: We heard about this before, so Brian will answer this question.

Dr. Brian Walitt: The answer is actually pretty simple, I think Nick did a really wonderful job talking about what EEfRT preference is for us, and the unconscious nature of effort in that aspect of it. The EEfRT task is framed as a measure of reward motivation, effort allocation or effort-based decision-making. These terms effort allocation and effort-based decision-making frame task performance as an entirely volition action.

We chose effort preference to reflect both the conscious and unconscious aspects that guide the moment-to-moment choices that are made during the effort task. I think we are running a little bit behind, so we have one more question.

Moderator: It seems notable that your study found no evidence for POTS in the sample as it appears to be present clinically, can you say more about the seeming dichotomy?

Dr. Mark Hallett: Say again, I didn't understand the question.

Moderator: The question is, it seems notable that your study found no evidence for POTS in the sample as it appears to be prevalent clinically, can you say more about the seeming dichotomy?

Dr. David Goldstein: This has to do with the fact that –

Dr. Mark Hallett: I didn’t understand. Repeat the question so we can all understand.

Dr. David Goldstein: Other groups think that POTS is very common, how can we didn't find that? I think that is the question. One, the facts are the facts, this is what we have. It's a small study, but I think mainly it's a matter of referral biased. There is no referral bias in this study, but when it comes to other groups who focus on autonomic disorders and in particular, POTS, when they are asked how frequent is POTS in chronic fatigue syndrome, since this is what people see, this is what they find. Yes, it's very common.

I think to an important extent, the referral bias could result in overestimation of the frequency of POTS in the ME/CFS population.

Dr. Avindra Nath: Let me just add one more thing to it, that's in the next session, I think you'll hear a lot about autonomic dysfunction, and I think it may not be classical, but there is enough autonomic dysfunction suggesting that there is dysautonomia in the patient population that we studied.

Dr. Brian Walitt: The last point to make is that a large percentage of the patients actually did have POTS. What makes it really different is not that the participants didn't have POTS, it's that the healthy volunteers did also, that a lot of them had criteria when you took them on a

tilt table test which shows you what is really different is that we had healthy volunteers and maybe POTS in clinically asymptomatic people is a higher frequency than people realize. It's not that the patients didn't have POTS, it's that the healthy volunteers did.

Dr. Mark Hallett: Thank you.

Dr. Avindra Nath: Maybe we can then now and this session and then come back after lunch? It is 12:17 PM. Let's give you a round of applause.

Dr. Avindra Nath: Should we come back, we are scheduled at 12:45 PM, maybe we can take an extra 15-minute break and come back at 1 PM? Okay, let's come back at 1 PM.

Dr. Avindra Nath: Let's get started, the next session is on bioenergetics and that will be led by Dr. Kong Chen. He is the Co-Director of the Metabolic Clinical Research Unit and also Chief of the Energy Metabolism Section, and the Director of the Health Energy and Body Weight Regulation Core.

His group developed advanced techniques such as whole-room indirect calorimeters that are used to measure the rate of energy expenditure at the minute-by-minute level, and substrate oxidation for several hours or several days.

He can simultaneously measure movement and physiological parameters in this well controlled environment to study the impact of physical activities, diets, medications, and environmental temperatures and energy metabolism, and hormonal responses. He led the all the bioenergetics studies here on ME/CFS and we look forward to hearing from him. Thank you.

Dr. Kong Chen: Thank you, Dr. Nath. I have the pleasure and I want to introduce some speakers but first let me introduce you to the concept of bioenergetics. You may have already heard some of the terms and about energy. In our view, energy is – I guess I should use this.

There we go, this is kind of an interesting system, it doesn't look like – nevertheless. You'll see this person on your right, and we talk about energy is the calories in or out. The calories can be directly measured by heat, or in our case, measured indirectly. As you heard from Dr. Nath, we measure the carbon dioxide production and oxygen consumption both in the context of the whole-room setting as well as in the exercise setting where you wear a mask.

So this energy, especially in the diet, on these chemical energy carbohydrate fats and others is taken in by the body and is converted to ATP. We can measure that, and Shanna will introduce you to some of the measures of energy intake, particularly the macronutrients. Then we will hear about how we collect the energy expenditure and represented metabolism.

We also go a little deeper, quite literally, we will measure the body composition which is the fat storage. Anywhere in the system, if they have problems, we may see the signatures. We have experience with patients with chronic diseases such as kidney/liver diseases, cardiovascular diseases, thyroid, diabetes, neuromuscular disease, and certain cancers, all show various bioenergetic signatures.

By identifying them we can reveal the mechanisms of pathophysiology and perhaps open treatment targets. So that is what the rationale is of studying this group.

As we have shown you, it is a stepwise process in both deep phenotyping visit, and as well as exercise stress test. Just like other groups, we will take different pieces of it, and you'll first hear from Barbara Stussman on the post-exertional malaise, so this is sort of the last session, you'll hear the best part. Barbara will present that. Shanna, with her collaboration with Sara Turner, will present the dietary evaluations.

Sam LaMunion from my lab will present the body composition data presenting the body fat mass distribution. He will also present wearable accelerometer data representing the free-living physical activity levels.

Then you will hear from another part of my lab, from Rob, and before that, you'll hear about the exercise testing itself. This is done in sort of the stress test measurements. That will be presented by Lisa Chin and Bart Drinkard, and Lisa will be doing the talking. As I said, Robert will present the whole-body expenditure data.

Last but not least, we will hear from Paul Hwang, who is taking the muscle samples and looking at the micro level of the endoplasmic reticular stress, or ER stress, and mitochondrial function.

With all that, and please keep in mind there are two key questions when we do these measurements. The first one, are there any phenotypic signatures in the whole-body energy balance measures? With the exercise challenge, are there any unique changes in these measures? With that said, Barbara, please stand.

Dr. Barbara Stussman: Thank you, Dr. Chen. I will give a brief overview of our research related post-exertional malaise or PEM that we published in a few papers. The first paper was a focus group study conducted to learn more about PEM and to inform the study procedures in the main study. The second paper describes our mixed-methods system to assess PEM at a singular timepoint.

So what is post-exertional malaise? According to the 2015 Institute of Medicine report on ME/CFS, they describe it as a "worsening of symptoms after physical, mental, or emotional exertion that would not have caused a problem before the illness. PEM often puts the patient in relapse that may last days, weeks, or even longer. The symptoms typically get worse 12 to 48 hours after the activity or exposure and can last for days or even weeks."

Dr. Brian Walitt and I met back in 2016 when the ME/CFS study was first being developed. We discussed how best use qualitative methods to study PEM. At that time, PEM was not well studied and there were no validated measures. That is how the focus group study was initiated, and I want to acknowledge my co-authors here.

The objectives of the focus group study were to inform the study design of the main study. Specifically, we wanted to determine individual PEM symptoms to quantify, and the specific timepoints for assessing PEM before and after the CPET. We chose focus groups because they are useful for underexplored topic areas, as was post-exertional malaise at the time. We conducted nine focus groups with 43 participants from November 2016 through August 2019 ranging in length from an hour and 40 minutes to two hours. They were conducted over the phone to allow participation from people all over the country who could not otherwise travel very easily.

So, in terms of the findings from our study, we found that PEM is triggered by three broad categories of events: cognitive effort, physical activity, and emotional triggers. You can see examples of these under each of these categories on the slide.

This word cloud shows PEM symptoms after CPET by the focus group participants. The size of the words are proportional to the frequency of the symptom being reported. As you can see, there were very wide range of symptoms, but there were also three core symptoms mentioned by virtually every participant: exhaustion, cognitive difficulties, and neuromuscular complaints.

Based on what the participants told us during focus groups, we charted the onset, peak, and recovery after CPET. Participants said that PEM onset occurred from immediately to 48 hours following the CPET. The peak of PEM ranged from immediately to a week following the CPET. Participants said they returned to their previous CPET levels from a minimum of two days after the test to some reporting that they never fully recovered. Some participants reported a longer recovery after the two-day versus the one-day CPET.

There were several implications from this focus group study. Based on these findings, we developed our own system for assessing PEM that we used in the main study. For our system we conducted qualitative in interviews at six timepoints before and after CPET, along the visual analog scales that were also mentioned earlier this morning. During the qualitative interviews we asked every patient how they were feeling physically, cognitively, and emotionally, as well as their most bothersome symptom at every timepoint.

Additionally, some patients described a more difficult recovery after the two-day versus the one-day CPET. Also, because focus group participants described the severity of the symptoms following the CPET, and the difficulty of the travel required to get to the CPET, we allowed all patients to rest for two days at the Clinical Center in order to reduce any potential harm.

Now I will talk about the second paper, which was published in February this year, detailing and validating our system for assessing PEM after CPET. Again, I would like to acknowledge my co-authors here.

For this study we analyzed transcripts from our interviews, and as I just mentioned we conducted these interviews at six timepoints before and after CPET, which generated hundreds of pages of transcripts for each participant.

Four researchers individually read each transcript, plotted the trajectory of PEM, the peak of PEM, and the most bothersome symptom for each participant. We achieved 100 percent consensus by reviewing each transcript, line-by-line together and having in-depth discussions to adjudicate any disagreements.

This is an example of how we used the qualitative interviews to plot a single patient's PEM. In this example, the patient started out slightly worse than the usual baseline before the CPET, got worse until they peaked at 24 hours, and then started to get better. The red dotted line served as a benchmark representing a severe episode of PEM the patient had previously experienced in their daily life. To meet the threshold for having PEM, an increase from the pre-CPET value and peak rating of at least 3 was required. A peak of 3 was chosen because it was clinically meaningful with disruptive symptoms. Based on this, none of the healthy volunteers in the study experienced PEM, and all of the ME/CFS patients experienced PEM.

Next, we overlaid the PEM interview data with the visual analog scales and the VAS scales provided a continuum ranging from "not at all" to "most extreme" and patients marked the level of their symptoms all on the continuum. Visual analog scales were filled in by patients at the same timepoints as our interviews. We also created a composite VAS that combined 12 of the individual symptoms.

This graph shows one patient as an example. For this patient, the fatigue VAS in black and the composite VAS in purple remain flat throughout the entire course, while the PEM interview rating in blue shows more granularity.

This table shows correlations between PEM interview rating in VAS scales. In the interest of time, I won't go through the whole table, but the main point is that when we singled out the ME/CFS patients most bothersome symptom, the correlations between the interviews and the VAS measures were stronger at all three data points: pre-CPET, peak of PEM, and change from pre-CPET to peak.

This highlights the potential for using the most bothersome symptoms to improve the assessment of PEM.

The implications from our mixed methods study are that using our novel assessment tool, all ME/CFS patients were determined to have PEM following the CPET. Due to the wide breath of PEM symptoms, a measurement system with the ability to synthesize multiple symptoms into a single holistic experience works better than just looking at singular individuals. Isolating the most bothersome symptoms seem to improve the performance of the VAS scale. Our mixed- methods approach seems to have promise and it's available to researchers to use and to improve upon. We are currently using this system in our PASC or Long COVID patients. Thank you.

Dr. Lisa Chin: Good afternoon, everyone. I will be taking us through the exercise challenge that was done as part of the study. You may have heard it referred to as an exercise stress test, cardiopulmonary exercise test, or CPET for short. For those of you who are not familiar with the CPET, subjects are requested to cycle on an ergometer at a requested cadence, and during this time, intensity is increased over time.

The exercise test is stopped when participants are no longer able to maintain the set cadence or requested cadence and the limited tolerance is reached. Seen in this image, you can see that there are several non-basic physiological measures that are being taken during this test. For instance, you have gas exchange that is measured – measuring oxygen and CO2 concentration at the mouth. You also have heart rate by EKG and muscle oxygen saturation by near-infrared spectroscopy that is placed on the muscle.

With all of these simultaneous measures happening at the same time during the CPET it really gives us an insight as to how the different systems work together to provide the necessary oxygen during exercise to the working muscle. You're talking about taking inspired oxygen moving through the lungs, being pumped by the heart, going through the vascular system, and being extracted and utilized by the muscle, but also talking about the reverse process of expelling the byproduct of CO2. All of this is being captured through the CPET.

I do want to highlight that there are two main purposes to the CPET in this study. It was not only a physical fitness assessment, but it also functioned as a standardized fatiguing stimulus. For post-exercise measures.

Here we see with the exercise protocol looks like, and there are different ramp rates that we used depending on the participant. These were based off of predictive fitness. Because we know that PI-ME/CFS participants had a lower exercise capacity, they were all given 15 watt per

minute increment. For healthy volunteers however, they could have a 15, 20, or 25 watt per minute increment instead. Just to highlight the level or increase in the work related to optimizing an 8-to-12-minute exercise duration for nearly everyone.

What you see here in this figure is gas exchange from a healthy volunteer female of 54 years of age, and also, along the x-axis is the work rate, and along the y-axis here is the oxygen uptake or VO2 that is measured at the mouth.

We see that with an increase or linear increase in work rate over time, there is an increase in oxygen as work rate increases. This is reflecting that more oxygen is needed as you have higher and higher intensities that you're working towards.

So right at the end here, what we are interested in at the end here, that reflects somebody's, or an individual that is performing the task, their peak capacity. That is reported as peak VO2, but we also have the associated work rate as well, shown here as peak work rate.

The other variables that are measured during the gas exchange include those of ventilation and VCO2, which is carbon dioxide output, and also ventilation determinants. These are used later to determine the anaerobic threshold or the AT for short. The AT is a point shown here in this figure where fatigue is imminent. Activities done below this AT level are theoretically activities that you could sustain without incurring fatigue. However, if you were to do activities and to sustain them above the AT, that is when the fatigue would develop.

Other measures that were taken during the CPET test also reflect the cardiovascular systems. Here we are looking at heart rate and you can see that there is also a steady increase in heart rate towards peak as exercise increases or work rate increases. We also took measures at the local muscle level. Here we are looking at muscle oxygen saturation, in this case you see a slight decline, or a decline that is happening because the muscle is extracting oxygen from the micro vasculature as exercise progresses.

Turning our attention to the results. Here we are looking at respiratory exchange ratio, or RER for short, and that is just the ratio of CO2 to O2. For us, sometimes you can use the RER to reflect whether participants give it a good attempt on the exercise task. For us you can see that almost everyone except one had the RER greater than 1.1, which means key performance on the CPET was achieved in both groups.

Looking further at the results, there was a trend towards a lower peak power in the PI-ME/CFS participants, significant lower peak VO2 as well as VO2 at the anaerobic threshold happening in PI-ME/CFS versus healthy volunteers. When you look at peak VO2 to that is expressed in the percent of the predicted, this is based off of age and weight, you can see the majority of these, almost all but one PI-ME/CFS participant was less than 84 percent of predicted VO2. Conversely in the healthy volunteers, you see that the majority of the participants were over 84 percent of predicted peak VO2.

This really, to summarize, it is lower capacity that was observed in the PI-ME/CFS at peak in the anaerobic threshold compared to healthy volunteers.

Let's look at it in terms of practical terms. Here, this is showing the metabolic equivalent of task, or MET, where one MET is the energy requirement sitting quietly here. Along the right, you will be seeing a lot of different common activities and their associated MET values over here. For instance, carrying a 15-pound load on level ground is five METs, meaning it is five times the energy requirement than at rest.

Also, down here, about 1 to 2.5 METs is where your daily activity, activities of daily living, would reside. These are talking about just basic self-care of just eating, grooming, dressing.

What is shown in the shaded area is the instrumental activities of daily living, that is 3to 5 METs and that is more complex functions to support independent living in the community. This is cleaning, cooking, taking transportation.

Let's look at the data from the study. Here we have on the left, anaerobic threshold, and on the right is peak exercise, but what we see as a group is that you have PI-ME/CFS participants having lower METs at AT and also at peak exercise. For these individuals shown here, below the 3 MET level for AT, performing instrumental activities for daily living will be difficult. Compound that if they were also having a MET level of less than 5 at peak exercise.

Knowing that there is about up to 6 METs from more intense activities, you can see what is still termed moderate activity, there is a fair amount of PI-ME/CFS participants, that would be reading at their peak limits performing those kind of activities.

Here is the heart rate data, we see here is a trend to lower peak heart rate, but expressed as a percent of their age predicted heart rate, you do have a lower number that is being measured in the PI-ME/CFS versus healthy volunteers. Couple that together with a higher resting heart rate, you actually have a lower much lower heart rate reserve in PI-ME/CFS compared to healthy volunteers.

What this means is, that chronotropic incompetence or CI was found in five of the eight participants for meeting less than 85 percent of their age predicted heart rate reserve was found. Then during exercise, this graph over here, you can see over the course of exercise, there was a lower than expected heart rate response among the PI-ME/CFS participants, but not among the healthy volunteers.

Just to wrap up in terms of ventilatory efficiency, so this is how well the lungs function to be able to expel CO2, there was no real difference between the groups. Neither was mechanical efficiency across the work rate, this was also similar between the two groups. The micro vasculature, this was looking at the muscle oxygen saturation, there was also no real difference between the groups, however there was a small sample set and there was a lot of variability. Again, similar ventilatory efficiency, mechanical efficiency, as well as muscle tissue oxygenation were found between the groups. Thank you.

Dr. Shanna Yang: Hi there, my name is Shanna and I'm a dietitian at the Clinical Center and I will be speaking about our dietary evaluation as part of the study.

Let's start by talking about why we looked at typical diet as part of the study. We were just getting started with the conceptualization of the study, we looked at the literature to see what was out there. Not super surprisingly there wasn't a whole lot out there describing diet in this population. There were some mixed reports on what was out there. Some publications mentioned frequent avoidance of various dietary items or food groups including things like dairy, gluten, sugar, which I think we have heard anecdotally.

Other studies that actually quantify dietary intake which they were very few, found that nutrient intake was not different between healthy volunteers of the general population, and the PI- ME/CFS group. So those mixed results led us to think that we should look further into this. The other reason is that there is a lot of interest in dietary intervention to help with managing symptoms of PI-ME/CFS. In 2017, a review article mentioned a whole lot of different intervention trials looking at diet, mostly focused on supplements, but also looking at diets like low sugar or low yeast diet, so because of this another reason we thought it was important to quantify diet.

How did we do that? We used two different tools. The first one is the diet history questionnaire, or the DHQII. This is a comprehensive food frequency questionnaire that covers dietary habits over a long term. Come in this case over the past year. It covers 134 food items and it's used to assess typical nutrient intake over the past year and it does quantify portion size as well. It is web-based and self-administered. It is completed by all participants at their first visit. This provided a pretty general assessment of dietary pattern over a long term period of time.

I know this is kind of small, but these are some screenshots of the questionnaire, so it follows logic and it asks about all of these foods, then if you say yes I do eat this food, it goes on to ask and further detail how often do you eat this food in the summer, how often do you eat it in the winter, what is your portion size, etc. It's very comprehensive.

The second tool we used where food records, and we asked each participant to keep a seven-day food record for the week prior to their second visit at the Clinical Center. This covered the same period as the accelerometer. When they came in, we reviewed this food record with them for accuracy and completeness and we verified pretty much every detail you can think of. Brand names, cooking methods, amounts, etc.

We probed for any missing or forgotten items. We then took this, and we coded this information into a software called Nutrition Data System for Research, or NDSR, to quantify dietary intake down to the nutrient level. This gives us some different information than the DHQ because it provides very granular data that is rich in detail and precision, but only covers a short-term period, so it doesn't look as much as the patterns as much as the precise detail.

Moving on to the results, this covers energy and macronutrients and overall, I think the take away here is that from both tools we use, we didn't see much difference between our healthy volunteers and our PI-ME/CFS participants.

As you can see, energy, there was no difference across the board, the only macronutrients looking at carbohydrate protein, fat, alcohol, that we did find a little bit of a difference and was saturated fat where we saw the percent of calories from that nutrient was higher in PI-ME/CFS when compared to healthy volunteers. We found that fiber was slightly higher in our healthy volunteers in comparison to the PI-ME/CFS population.

Moving to the micronutrients, this is looking at the minerals and vitamins, and this looks at only dietary intake, this does not include any supplementation being used. Again, we didn't see much difference in terms of intake, the only difference that we found was a slight difference in terms of folate intake with healthy volunteers eating a little bit more.

Switching gears, another thing that we did in terms of diet as part of the study was during the second visit, we actually controlled diet for a part of the study, so they were no longer eating their typical diet, they were eating what we actually provided to them. There were two real reasons we did this, the first was, as you have already heard, whole body energy metabolism is being analyzed in respiratory chambers. As Dr. Chen explained when we were looking at energy out, we need to be able to quantify energy in very precisely. In order to do that, we need to know exactly what people are eating.

For this reason, we controlled their diet during the period of time while they were in the respiratory chambers. When we were doing that, it's also really important to control diet so we could potentially isolate individual metabolic factors and know that it's something related to the individual metabolism versus the fact that perhaps someone's eating high carb and someone is eating high fat, or some difference like that.

You'll see in the picture below, that is our research kitchen and that is one of our cooks preparing what we call a metabolic diet. That is how we control diet while metabolism was being assessed. We provided a diet that was controlled in a number of different nutrients, every food and beverage was weighed out to the exact gram. As planned, the diets were controlled for energy, those were calculated to meet individualized estimated energy needs using a standard activity factor.

Macronutrients were standardized across all participants with 30 percent of energy coming from fat, 15 percent coming from protein, and 55 percent coming from carbohydrate.

Participants were then asked to consume that diet in entirety, and any refused items are items that people were unable to finish, were then weighed back so that we could calculate the consumed diet data to take into account. You'll hear little bit more about this as when you hear about the results of that respiratory chamber. Thank you.

Dr. Samuel LaMunion: Good afternoon. My name is Sam LaMunion, I will present to you now on body composition measures and energy. Very briefly I'm going to talk to you about the relationship between energy expenditure and body composition. As you imagine, body composition is the makeup of tissues in your body including bone, lean, fat masses. You might wonder how does body composition relate to energy balance and energy metabolism? Fat-free mass, or lean and bone tissues, accounts for between 70 to 80 percent of resting energy expenditure which you can see in the figure on the right which you'll see again momentarily from Dr. Brychta. Resting energy expenditure accounts for between 60 and 75 percent of total daily energy expenditure but this varies by individual.

How do we measure body composition? You see a picture on the right. The GE DEXA scanner which we use on the 7th floor of the southwest metabolic suite in this building, and we use dual energy x-ray absorptiometry or DEXA for short. Patients completed a whole-body DEXA scan at the phenotyping visit. From the DEXA scan we were able to get output on whole-body and regional tissue composition measurements of fat mass, lean mass, bone density, and estimated visceral fat, which is the fat around your vital organs.

The reason we did this is our question was what does body composition look like in PI-ME/CFS compared to healthy volunteers? Shown on the right is an image of a DEXA scan from not anyone we studied, just to give you an idea of what the image output looks like.

What we found was that body composition was normal in PI-ME/CFS, or at least not different from the healthy volunteers that participated in the study. You'll see here in all the panels and panel A that lean body mass was not different, no statistical differences at least for all the measures we have for lean body mass, fat mass, bone mineral content, body fat percentage, visceral fat mass, which again is estimated, then regionally dominant arm, lean mass, and legs lean mass. One thing I want to highlight here is in both groups, both healthy volunteers

and PI- ME/CFS, you see a lot of individual differences which is expected when we see this in the population, as Dr. Chen highlighted in his introduction. We see a lot of patients with chronic diseases and a lot of healthy volunteers and both groups here are effectively correlating with what we see in different populations across individuals.

The next part here, what is the relationship between physical activity and energy expenditure? I will highlight the figure on the right which I have pared down a little bit for you. The top part there is physical activity or activity energy expenditure and how does that relate to total energy expenditure. In addition to resting energy expenditure in the thermic effect of eating, which will hear about from Dr. Brychta momentarily, the last part there for activity energy expenditure makes up between 15 to 30 percent of total daily energy expenditure, of course there's high individual variation both day-to-day and across individuals.

This could be estimated from our wearable device data. I want to highlight here what you see in this figure, you see it's split into two categories there, exercise energy expenditure which is purposeful, you're going for a walk or a run, or participating in a structured activity. Or you are doing a non-exercise activity, thermogenesis or incidental activity, activities of daily living, as highlighted by Dr. Chin in her talk, these are things you're doing when you're milling about the office, transportation, that sort of thing. All that together can be measured with accelerometer which is what we will look at next.

As Dr. Yang highlighted in her portion of the talk, we did assess free-living physical behavior or habitual physical behavior for the seven-day period between visits, this is aligned with the outpatient diet food records. Our question was what does activity look like in PI-ME/CFS and was it any different from healthy volunteers? The way we assess this was using triaxial waist-worn accelerometer. There's a picture here, it's a small portable device, not transmitting any data, it's all recorded locally on the device. This is the device measuring movement in three planes and no other physiological outcomes, no other parameters, just movement.

This data was collected at 80 Hz and later collapsed to one minute epochs or activity counts. We required them to have at least seven days of measurement, and this needed to be from valid data of at least 10 hours a day of wear time between noon and noon. This was standardized, measures from people that didn't wear it as much, those days got discarded or at least not included in these analyses. We used a validated cut point for estimating moderate to vigorous physical activity, one of our outcome measures shown on the right. First, we will look at mean total steps per day, and what we did see was that the PI-ME/CFS group did take significantly fewer total steps per day compared to healthy volunteers.

In the middle, this is the mean vector magnitude total activity counts and what the vector magnitude means is the square root of the sum of the squares, and you don't have to worry about the math, it is a triaxial summary measure of the numerous counts per day. This is an integrated summarized metric, so more counts effectively translates to more movement.

This is another volume measure, and we saw that the PI-ME/CFS group had less total movement per day for this measure.

Lastly, for intensity measured with which Dr. Chin highlighted in her talk, moderate intensity physical activity is used as a general outcome for assessing physical activity in free-living, so this is starting at 3 METs, and this is an absolute cut point for everybody, or at least adults. What we saw here again was that the PI-ME/CFS group did accumulate or engage in significantly less moderate intensity physical activity on a daily basis. Again, like highlighted with the body composition measures, within each group we did see quite bit of variability. This does, again, varies within individuals, day-to-day and of course, within individuals in that group.

With that, I'll turn it over to Dr. Robert Brychta.

Dr. Robert Brychta: Hi, I am Robert Brychta, a Staff Scientist at NIDDK. I will be talking about our measurements of whole-body energy expenditure.

I presented a little diagram about what we used to measure the whole-body energy expenditure which is called the room calorimeter, respiratory chamber, or metabolic chamber. Here, we see in white is the room, and in yellow is the air buffer around the room sort of a corridor around the room. Fresh air can go into the room through only a small portal, and the rest of the room is sealed. People stay in the room, and they change the composition of the air by consuming oxygen and producing carbon dioxide. We pull air out of the room, and so there is a one-way flow of air and we measure the flow of air out of the room, and sample the composition of the air using an oxygen and

CO2 analyzer to measure the concentration of the air over time and we compare what is going on inside of the room that is getting changed by the subject to what is going into the room, and the fresh air, composition of the oxygen and CO2, which in the fresh air is pretty stable.

Now, here is an example of our metabolic chamber at NIDDK Bethesda, people spent from – in this study, people spent from about 4 PM until about 8 AM, and the number of nights varied but most of our participants spent about four nights, four to five nights, in this metabolic chamber. It looks like a walk-in freezer, if you'll notice. I don't know if I have the pointer, but in the top there is the sampling ports which are metal, and then they are attached to the motion sensors so we could tell if people were moving or if they were not moving and asleep.

As Dr. LaMunion pointed out, this was, we can measure all of the components of energy, daily energy expenditure using this device, but since as I mentioned, the time constraints of the people's schedule when they're doing this intensive study. We measured them, these particular subjects, from 4 PM until 8 AM. Basically, we are concentrating on measurements of their resting and sleeping energy expenditure, and particularly their response to the CPET.

To do that, across the people, healthy volunteers and patients, we minimized the variability of their activity energy expenditure so they remained sedentary or asleep during the study, so they basically, when they weren't asleep, they were just watching TV and relaxing.

Then we minimized the variability in what is called the thermic effect of food or their energy response to breaking down the food of their diet. I think each person got one meal in the chamber, but as Shanna alluded to, the meals are standardized and the diet is a weight maintenance diet specifically for each person's needs.

This is just a tracing, a 24-hour tracing of what we normally see in the metabolic chamber and this is not a person in this study, it's a different study, but you can see at the end there is the sleeping energy expenditure highlighted in red, and this is the most stable component of your daily energy expenditure and has the lowest minute-to-minute variability. It is the most reproducible from night-to-night within a person and it's highly correlated as Dr. LaMunion said, to your fat-free mass. Your resting energy expenditure and sleeping energy expenditure is explained by about 70 to 80 percent of the variation and that is explained by core fat-free mass.

So this is difficult to measure without one of these room calorimeters because you can imagine, if you're measuring oxygen consumption of CO2 production to get energy expenditure, you would have to wear a mask or something else and that is really difficult to sleep in.

Here's what we found with our sleeping energy expenditure and this is at baseline the night before the CPET, and here, again the PI-ME/CFS patients are in red and the healthy volunteers are in blue. The women are the circles, and the men are the squares, you can see in general, women have a lower fat-free mass than the men they are on the left corner of the graph and that is because women are generally shorter than men, so they will have less fat-free mass. But you can see that across the whole group, the fat-free mass and sleep energy expenditure is highly correlated, and there is not really much of a trend between that differentiates the healthy volunteers and the PI-ME/CFS participants.

You can see here the R squared is 0.78 so it is about 78 percent of the variation explained by the fat-free mass. When you look at total energy expenditure, again, it's only from 4 PM until 8 AM, again, they are mostly resting so most of the time is sleeping but again, it's explained by about 75 percent of the variation is explained by their fat-free mass.

When we look at the sleeping energy expenditure from before the CPET to after the CPET, this is the night after the CPET, that is in the open circles and open squares, and there is a line connecting each participant to their own baseline measurement before the CPET to after measurement. You can see that the red dotted line, which is the regression of fat-free mass to sleeping energy expenditure after the CPET, basically falls right on top of the one before the CPET. There wasn't really much of an effect, and you can see that as this graph as each person compared to themselves before the CPET and after the CPET. Again, healthy volunteers in blue, PI-ME/CFS in red and there really wasn't much of a difference between before and after, there is no trend anywhere.

In the total energy expenditure again, we see the same situation where the regression lines of fat-free mass versus total expenditure fall on one another and comparing the person to themselves, we don't see really any difference from the night before to the night after the CPET.

The other thing that we can look at with the metabolic chamber is called the respiratory quotient or I think Lisa referred to it as respiratory exchange ratio, and this is your measure of carbon dioxide expired to oxygen consumed. When you are exercising, this is a measure of your effort or your, how much you are expending. When you are at rest this reflects what fuel you are using and where the majority of your energy is coming from. A ratio of 0.7 would be purely fat in the diet, and 1.0 would be purely carbohydrate, and then the standard mixed meal is someplace in between that.

We can see at rest, or at sleep, there was really no difference between any of the people compared to themselves in their sleeping respiratory quotient, which is a little bit lower than their respiratory quotient while they were over the whole time, while they were awake and had a meal. So again, there is really no, there was no detected difference between the night before and the night after the CPET in that measure.

With that, I will give it to Dr. Hwang.

Dr. Paul Hwang: I am Paul Hwang, an investigator in the Heat, Lung, and Blood Institute. I will start out by thanking the meeting organizes, especially Dr. Nath and Walitt for giving me this opportunity to me to present our work. What I would like to do today is share with you a project we have been working on over the past several years trying to determine the molecular mechanism of how there might be a deficiency in the skeletal muscle of patients with ME/CFS.

We had reported originally early in our studies at NIH that individuals with Li-Fraumeni syndrome, which is a cancer disorder caused by germline mutations of p53. Germline mutations of p53, that is again, an early onset cancer disorder, have increased metabolism of mitochondrial function and actually increases exercise endurance.

A patient had read our work on the internet and emailed me saying that she was the exact opposite of what we had reported and that she was always fatigued and had terrible exercise endurance. This is the case of a 38-year-old woman with Li-Fraumeni syndrome and fatigue symptoms that had started since her teenage years when she contracted mononucleosis, an infection resulted but had symptoms that never went away. She had had extensive clinical workup, medical workup, that had mitochondrial disease evaluation with mitochondrial genome sequencing, which were all relatively unremarkable.

She had the typical symptoms associated with ME/CFS, and so as many of you already know, there's been many different mechanisms proposed for ME/CFS. Some include dysfunction of mitochondria, but no specific mediator has been identified.

We had studied over 50 Li-Fraumeni syndrome patients at NIH, none of them had reported fatigue symptoms, so we were rather intrigued by this report, and we decided to study the patient. We invited her, and she has indicated here as a sibling S1. Basically we did a phosphorus-31 MR spectroscopy study, a noninvasive test of the leg exercise muscle trying to – it's been shown by other investigators as a marker of mitochondrial marker of mitochondrial dysfunction.

When we performed this test on the patient, we were amazed with how strong her recovery time constant was at 80 seconds over two different time periods of six months. We were fortunate to be able to invite the brother sibling, S2, who also had Li-Fraumeni syndrome, but he didn't have the fatigue symptoms. His phosphocreatine recovery time was 30 seconds, and that is in the realm of what we had previously observed in other Li-Fraumeni syndrome, and that is actually faster than non-carriers who are in the realm of 36 seconds.

We were able to obtain biopsies from both individuals and made fibroblasts and we did a very simple experiment just looking at oxygen consumption as an assessment of the mitochondrial function. You can see that the patient, S1 oxygen consumption both in the basal and maximal state was significantly lower than the brother’s cell oxygen consumption.

This was a very interesting finding, and we pursued this further and basically just to summarize a large body of work, we were able to identify increased WASF3 overexpression in the patient’s cells as being a potential mediator of the mitochondrial dysfunction. This really, finding this really involved tracing this learned signaling that we were observing in p53 phosphorylation and p38 kinase activation. In retrospect, it turns out that the signaling abnormalities were all secondary to a primary defect in mitochondrial function in the cells.

We identified this gene called WASF3, which stands for Wiskott-Aldrich Syndrome protein family member three, it's the gene that is mutated in this immunodeficiency – it is a family member of the gene that is mutated in this immunodeficiency syndrome.

Interestingly, it had been reported a number of years ago that WASF3 was a top candidate gene associated with ME/CFS in a large bioinformatics meta-analysis study. But not much more had been done subsequent to that report. It was a well-studied gene, it's known to interact with actin involving actin polymerization for the cytoskeleton of the cell. Also known to play an important role in scaffolding protein complexes. Because of this, it's also known to be important for cell migration and has been studied in the cancer field.

We wanted to examine function, so one of the early experiments we did was to simply downregulate WASF3 protein levels in the patient cells, again the S1 patient cells. Using a simple shRNA, and are happy to see that there was a significant improvement in oxygen consumption when we knocked down the levels, decrease the amount of protein WASF3. Associated with that was a nice increase in the mitochondria respiratory complex for proteins, such as COX1 and other proteins as you can see here.

We also did the opposite experiment of overexpressing WASF3 in myoblasts, you can see when we overexpressed the protein, the oxygen consumption was decreased and associated with that, you can see that when we do, look at the different respiratory mitochondria respiratory complexes, complex four subunit protein such as CO1, COX1 subunit, is significantly decreased while the other respiratory complexes such as complex five, three, one, proteins are not changed.

The reason for this becomes clearer based on further mechanistic studies that we did and basically again, to summarize our work we show that WASF3 protein is indeed localized to the mitochondria and enriched in the mitochondria, I should say. Using a blue native gel and proximity ligation assays, which allows us to look at the molecular interaction between WASF3 and some of the mitochondrial respiratory complex, we are able to show that WASF3 can interact with the respiratory complex three subunit proteins, a couple of specific subunit proteins. When WASF3 is overexpressed, it disrupts the assembly of the super complex, the complex three, complex four proteins. It is well known in the literature that when you destabilize complex four proteins, that they get degraded.

So that sort of explains what we observed with the protein levels, when WASF3 is overexpressed, subunit proteins go down. Besides making a mouse model where we overexpressed WASF3 and ensured the significant decrease in exercise capacity and in fact having exercise capacity in mice on a treadmill, we had great opportunity to obtain samples from the ME/CFS study. And we looked at the skeletal muscle biopsy samples that were stored away from ME/CFS patients compared to healthy volunteers. We observed very nice significant increase in the protein levels in the muscle samples of the ME/CFS patients.

You can see here the inverse relationship within the WASF3 expression and the complex proteins as well, again from the muscle cells. In terms of what might be making the increase of the WASF3 protein levels, there is evidence in the literature that ER stress is involved in increasing WASF3 protein levels. We looked at one of the markers of the stress called PERK protein, you can see that overall there is a trend of increase expression of ER stress protein marker compared to the healthy volunteers.

Most importantly, we were able to take the patient cells, again the ME/CFS patient, put the cells in culture, and we added specific stress inhibitor called salubrinal, it works specifically through inhibiting a phosphatase that is involved in regulating ER stress. And we were able to show here that in a drug dose dependent matter, that we were able to recover the mitochondrial function of the patient cells, as you can see here.

In parallel with that, we were able to show recovery of the super complex, complex three dimer plus complex four subunit proteins were increased in the patient S1 cells by the addition of the drug, the chemical salubrinal, while the protein levels of complex four, for example, was unchanged. Again, that speaks to what I showed you earlier with the specificity of the interaction that seems to be localized to the complex three protein.

Basically, just to summarize, I hope I'm able to show you that patients with ME/CFS have increased WASF3, at least in a subset of the patients, and that WASF3 molecularly disrupts respiration by preventing super complex formation, destabilizing complex four is induced by ER stress, inhibiting ER stress improves mitochondria function, suggesting potentially treatment strategies that would, I would like to investigate in the future.

Dr. Kong Chen: So, I have this easy job to put it together. So, let me remind you about 45 minutes ago when we talked about bioenergetics and you heard from a different aspect of the bioenergetics both in the scope of the whole body as well as in the last case, in the muscle cells. When they measure oxygen consumption, it's not in the chamber, it's a small or tiny chamber.

You can see that how we try to fit all of that in the sort of last part of the figure, so the peripheral, everything we study is below the neck except you Robert, you study the brain level. And it is reflected in the PEM.

The key question one we asked, are there any phenotypic signatures in the whole-body energy balance measures? And this is really compared to the healthy normal controls. If you remember the data, there were no detectable differences in habitual total energy or macronutrient intakes, carbohydrate, fat, and protein, and a couple of others. Maybe some slight trends in less fiber and folate, but more saturated fat intakes by the patients with PI-ME/CFS.

There were no detectable differences in the measured output on total energy expenditure both in sleeping as well as 4 PM until 8 AM. The substrate oxidation, is also very comparable between the two groups in the baseline and as well as after, one night after, the CPET.

There were no detectable differences in body composition, in bone, lean, and fat masses. The patients with PI-ME/CFS had significantly less free-living physical activity. It confirms the other both self-reports as well as the literature.

One thing that struck us the most is that the problem that has no surprises that there is considerable between-individual differences, particularly in the CPET test. We see a wide variety and for some, a couple of patients, had one of the higher oxygen capacities.

When it comes to question number two, are there any unique changes in these measures after the CPET? This is both compared to themselves as well as compared to the patients versus controls. Remarkably, there were no detectable differences in the ventilatory efficiency, mechanical efficiency, and muscle tissue oxygenation, measured in the CPET process but significantly lower peak VO2, anaerobic threshold, heart rate reserve, and heart rate response during the CPET.

There were no detectable differences in total or sleeping energy expenditure, nor in substrate oxidation rates after the CPET.

Again, there was considerable between-individual differences in CPET and PEM patterns.

Our interpretation of putting all this together, is that the patients with PI-ME/CFS have a wide range of bioenergetic capacities. The fact that we couldn't assess them before they have the symptoms, or the disease is we can't really tease out what is causing it versus what is the effect of it. While the whole-body energy balance appeared to be well-preserved at resting and following exercise challenge to reduce peak capacity and/or chronotropic incompetence, skeletal muscle mitochondrial dysfunction was evident, at least in some of these patients.

So, let me ask the panel back down, so these are the people if you have any questions specifically to these areas, we will be happy to answer them.

Joe Lanson: Okay. Joe, it's definitely on now. Joe, I'm a patient. It was stated in the first presentation that there was no validated measure for post-exertional malaise. Two questions, what would a validated measure for PEM look like and why does the two-day CPET not qualify as a validated measure given its currently being used, as I understand it, for disability evaluations? Thank you.

Dr. Barbara Stussman: At the time that we started the focus group study back in 2016, at that time there were no validated measures of PEM. There is no validated questionnaire to assess PEM, but to my knowledge, no validated measures to assess PEM at a point in time before and after CPET. There is the VAS scales that have been used traditionally but have not been validated as such for that specific purpose, but I will let Dr. Walitt answer this.

Dr. Brian Walitt: The whole questionnaire was validated as an instrument to measure the existence of PEM in a person after we started. So, there really wasn't any instrument while we were starting, and right now, that would be sort of like the go to that most of the studies are using and it's been really used in Long COVID research as a measure of PEM.

In terms of the two-day CPET, that is really measuring further collapse of metabolic activity, it's not really measuring the experience of post-exertional malaise, which is a delayed response to exercise that somebody feels. So, what might be physiological measurement really isn't capturing what that experience of PEM is, and in that way the two-day CPET is not really a measure of PEM but a measure of cardiorespiratory performance for the second exercise.

Dr. Avindra Nath: Also, isn't it true that by the second day, after the first day CPET, these patients are really pretty exhausted and putting them through a second day would have been tough –

Dr. Brian Walitt: Yes, with Barbara's instrument, we were able to demonstrate that everybody had PEM after one day of the CPET. So the two-day CPET wasn't necessary to put them into PEM.

Dr. Avindra Nath: We could potentially have hurt them.

Dr. Brian Walitt: Yes, we could have.

Moderator: We have a couple of questions from our online audience. The first one is, have the findings WASF3 in mitochondrial dysfunction been seen in Long COVID patients or fibromyalgia patients?

Dr. Paul Hwang: That is a great question, we are very interested in doing that and actually, we are of course, collaborating with Dr. Walitt and Dr. Nath on Long COVID. We would like to definitely look at that, so that would be great to look at.

Fibromyalgia, we haven't looked at it, but it would be great to look at it in some of these methodologic disorders that are associated with fatigue, those are all great things to do, and I hope there would be others out there that have read our paper that will also do these studies.

Moderator: What do these bioenergetic studies tell us about the nature and cause of the further lowering of anaerobic threshold seen on the second day of the two days CPET test in ME/CFS patients?

Dr. Kong Chen: So, can you repeat the question, on the second day of CPET?

Moderator: Yes, what do the bioenergetic studies tell us about the nature and cause of the further lowering of the anaerobic threshold seen on the second day of the two-day CPET test?

Dr. Kong Chen: We didn't do a second day CPET test, it would be hard to speculate what may be related. Mark?

Dr. Mark Hallett: I have a question, I wonder if you could comment on chronotropic incompetence, what is the nature of that? That's one other element of this, and I wonder if we have any explanation for it?

Dr. Kong Chen: Lisa?

Dr. Lisa Chin: At least from the measurements of the heart rate, and we were looking at it based on the percent predicted, age predicted heart rate reserve, it's a cut off and that was, there were 5 out of 8 that showed it was that they had met the criteria for competence. We looked at it during the exercise duration or the exercise test, we could see that those slopes were lowered, there is a lower heart rate response across the different work rates in PI-ME/CFS versus the healthy volunteers.

So, whether this is a deficiency or related back into some of the autonomic dysfunction we are talking about, related to that, but that is what we were finding.

Dr. Mark Hallett: The finding was clear, I'm asking about what the explanation was. Where does chronotropic incompetence come from?

Dr. Brian Walitt: I don't really consider myself an autonomics expert in any way shape or form, however, chronotropic incompetence is thought to be a dysautonomia, so the autonomic system isn't kicking in as it is supposed to raise them up. David, do you have something to add?

Dr. David Goldstein: There is a systematic approach to chronotropic incompetence that was far beyond –

Dr. Brian Walitt: Do you want to come to the microphone? This is why we have a whole team of people here.

Dr. David Goldstein: There is a systematic approach through chronotropic incompetence, and it is beyond the scope of what was done here, but it boils down to dose response for isuprel that is infused, it is a catecholamine. So, you can measure the increase in heart rate for a given isuprel level, but more importantly, isuprel releases norepinephrine from sympathetic nerves by stimulating beta 2 receptors. By measuring the catechol response to isuprel, you can pinpoint the basis for chronotropic incompetence at least from the point of view of beta receptor mediated processes.

Dr. Brian Walitt: When you say that the findings, decreased norepinephrine that you found, could be related to why we are seeing chronotropic incompetence in these individuals?

Dr. David Goldstein: That is a misstatement because what we found in terms of norepinephrine is in the spinal fluid, which is independent, quite independent, of the norepinephrine in the plasma as a measure of sympathetic outflow, if you will, is a very powerful blood brain barrier for catecholamine. What you see in the CSF is either very indirectly related or not related at all to what you see in the periphery. By way of reminder, what we found in the periphery was the norepinephrine was normal. Norepinephrine is supposed to increase when you are tilted and it did increase and increased normally in the ME/CFS group. There is data about the sympathetic responses, sympathetic noradrenergic responses in this study are, let's say, inconsistent.

What we found is, we didn't see any evidence of a sympathetic noradrenergic problem in this study.

Dr. Brian Walitt: How about the increased epinephrine levels in some of those participants?

Dr. David Goldstein: As I showed in my slide, although you think fight or flight, get them catecholamines pumping and all, it's not the same thing. Especially in the setting of fainting reactions, we are tilting invoking sudden hypertension, really mediated the hypotension, there is a disassociation between what happens to norepinephrine and what happens to epinephrine, we saw that a little bit if you remember those individual spaghetti kind of plots.

Not overall in the group, you have to keep in mind that the autonomic nervous system and the sympathetic nervous system is not one thing.

Dr. Brian Walitt: I'm not sure if that answers your question, Mark.

Dr. King Chen: I think Mark is asking a critical question but let me answer by Brian's earlier remarks that this is a hypothesis generating study. You are asking the test, other than Paul's study of getting to the mechanism of skeletal muscles, I think we are just getting started.

Dr. Joe Breen: This is Joe Breen from NIAID. Paul, I have a question for you. We saw earlier, Brian's 'omics analysis saw clear differentiation between genders for the PI-ME/CFS group. Did you see that with your results?

Dr. Paul Hwang: Yes, so we had a mix of samples. We didn't really see a good correlation, the only, we looked at 14 individual samples with ME/CFS. More women samples than men but we didn't really see a clear correlation. There was quite a spectrum of expression levels, so I can't really comment on that.

Dr. Kong Chen: I believe we are running out of time, if there is any other questions, we would be happy to address them over email or other communications unless there's an urgent question.

Joe Lanson: Joe, a patient again, any guesses Dr. Hwang on what is causing the endoplasmic reticulum stress upstream in the mitochondrial dysfunction?

Dr. Paul Hwang: That is another great question. You have to remember that what we observed, the initial finding was in skin cells of the patient, it is fibroblasts, nothing to do with muscle, but considerably activating the stress in the cells compared to the brother sibling that didn't have that.

We don't quite know what it is, but if I was to speculate, it may, beyond stress is a response abnormal protein homeostasis in the endoplasmic reticulum and it's a stress response you see with infections, with viral infections. The cell tries to shut down production of viral proteins, so that could be one mechanism and the fact that ME/CFS is associated with a post-infectious state. Whether or not I can speculate those abnormal activation in stress, and the failure of inactivation by some mechanism, that we have no idea, but I would be grateful to have experts in the field because there are a lot of experts in that field and it would be great to have them study that as well. That is a good question. Thank you.

Dr. Brian Walitt: I will keep things moving here. I'm going to talk now little bit about data sharing and ongoing work to come. So, one of the, I think most important things that we can do as a research team is to follow the emerging attendance of open science, as the UNESCO recommendation on open science are here. In order to make things open, available, and inclusive to other people and even people outside of traditional science.

The major principles are to make things openly available, accessible, and reusable to everyone to increase scientific collaborations and sharing of information for the benefit of all. To open the process of scientific knowledge creation to everybody that is interested in being involved. There are five tenants of open scientific knowledge, and I will talk about the ones that are really applying to us here today.

The first being, make scientific publications available to all. If you don't realize that it cost a lot of money to make a scientific publication widely available. Typically, to access these things you have to be part of a university or have a friend that can get you a copy of the PDF from another friend, and this is, it really gets in the way of sharing information. We really understood that and made sure that our publication is openly accessible, and anybody can just go online on Google and type in Nature and Walitt and you can get the whole article that easily.

Second, is open data to everybody. We really went, bent over backwards to make sure that all of our data is available. This is our data delivery statement in the paper itself, which is multiple paragraphs, but I will make this a little easier. By sort of talking about what the contents are and where to find our data. All of our data is CC BY 4.0, through Creative Commons, most people aren't familiar with Creative Commons but it says when you share data, how are other people allowed to use it and what do you need to do.

With our data, everyone is free to share it, copy it, redistribute the material anyway that they want. If you can find commercial value in it, have fun. You can adapt the data, remake it, transform it and build upon even if you're looking to make commercial success through that. We won't revoke those freedoms. The only thing that we ask in return is that you attribute us as a source of data. If you're going to use our data, just give appropriate credit for that data and where it came from.

Our data is mainly placed in a new database that was developed by NINDS for ME/CFS research called mapMECFS. It is hosted by RTI and part of the ME/CFS Research Network. Lots of different groups are starting to place their data in this secure and flexible data platform, there is some data exploration tools that are embedded in the platform and our datasets can be found here, this is sort of what it looks like.

What we did for all of the people who are interested, is we recoded our coded data to use these new mapMECFS data identities. So, all of the data identities in our datasets are the same. If one wanted to look at the correlation between anxiety and norepinephrine, you could take both of those datasets and do that on your own. You don't need us or to work through us to do that, to work. We're going to be trying to use these mapMECFS IDs across all of our data sets even on other platforms.

If you're interested in learning more about mapMECFS, here's the email and the website for you.

Our raw data is stored in classic raw data archives, gene expression data is in GEO, sequence reads are in SRA, and our metabolomics data is in the Metabolomics Workbench and here's all the information if somebody wants to see the websites themselves.

Our neurophysiology data is from the fMRI or TMS, and EMG data can be found in the Pennsieve archive, which is a newer archive also funded by NINDS. That is developed to host this complicated types of datasets. If you wanted to look at our MRI data or TMS data, this is where you would go.

Coming soon, we will host all of our polysomnogram data, particularly our EEG data and actigraphy data in the national resource pictured here. The NSRR hosts a lot of different polysomnogram data and has all the tools embedded in it to do the research and the analysis that

people are interested in. Here is some more information about, all the information on other types of studies that can be found on the national sleep resource.

The next tenant of open science is code availability, and I would like to take a moment to thank Sam LaMunion, because he really put an unbelievable effort that was required to get all of our code together and re-analyze our data to make sure that everything was valid.

The data that was used in our paper, each of the figures has its own individual data source file that can be queried. Each table has its own individual data file to be queried, so you can go back and see if you can do reproduce all of our data and the data files are linked to the paper itself. Our Github repository, which can be found here, you will find all of the different codes used by all of the different analyses in the paper, all of the code where possible is in R and linked to the datasets themselves. You can see how we came to our conclusions and how we did our analysis.

You will find the code, from John McCullough, code we used for the PCA and RsdA analyses that I presented earlier, all within our code repository in an effort to try to make it totally understandable how we can come to these conclusions.

I will take a moment to talk about value added, what you see is our first broad pass of all the data. The big first paper. This doesn't mean that there can be more efforts and more things happening. We already talked about three other papers that sort of came about as we were just trying to get this paper out the door. Barbara Stussman, both her papers about post-exertional malaise, and Dr. Hwang's paper was about WASF3, and what few people understand is that there's been other papers with our data as well.

These three papers are really about autoimmune myositis, which is interesting to me as a rheumatologist, really come because of the value of our healthy volunteer data. That there is going to be extra value, not just from our ME/CFS data, but the healthy volunteers also can contribute to increase scientific knowledge by serving as a really well-characterized cohort for comparison.

We have a lot of ongoing work currently worth talking about. It was already mentioned that we have a detailed characterization of the gut microbiome that I have to sit down and look at carefully over the weekend. We also have plans to study post-exertional malaise more deeply, we have biological samples collected at multiple timepoints, and we are going to be running them through a multiplex analysis very soon, the plates just arrived this week.

There is a wide potential for studying sleep deeply, we only talked in broad strokes about clinical sleep disorders, but we didn't do much with looking at sleep microarchitecture, or circadian rhythm analysis, and the same with our actigraphy data. There's a lot of potential to study that further.

The data, once it is available publicly, whether we will do it or people in the outside world who are interested want to work with us, we are happy to go either way. There will be an expansion of our neurophysiology analyses, we have work already being developed to look at the multimodal neuroimaging of our physical fatigue while we showed the TMS and the fMRI data already, there is EEG data and ways to sort of combine all of these into unique understandings.

Also, I know that our colleagues that presented today are working on connectivity analyses to help understand how these norepinephrine rich brain areas may be playing a role or expanding on those initial observations that we made. We also are planning on doing some additional computational modeling, we have, as mentioned before, we are working with the VA, we are the first VA-Intramural NIH collaboration to study disease over here at NIH.

A project IN-DEPTH is that effort, and working with Nancy Klimas and her computational biology team and we are going to use some of their modeling work on our data. To look at immune signaling networks using the Elsevier's Pathway Studio and to use their drug target selection process by cross-referencing our network analyses with pharmacogenomic databases.

Lastly, we have a whole new world of work that is going on in Long COVID using a lot of the same methodologies that we presented to you here. One of the big questions people have is, is Long COVID and ME/CFS the same thing, are they different? When we collect enough samples and data, we may be able to answer that by comparing the data we presented to you today with the new data that we are generating.

I want to thank everybody, so many people were a part of this project. On the left are all the authors, all 75 of us, all who contributed ways large and small over the eight years that we did this work. I want to thank all of the people on the right to acknowledge them and their contributions, from Francis Collins to initiating this project, and Walter Koroshetz for supporting us all of these years, to all of the adjudicators listed here. And all of the people that were part of the project that came and go, and some who had passed away over this time.

Of course, our eternal gratitude given to the volunteerism of all the post-infectious ME/CFS participants that underwent this entrusted in us to do this work with them and healthy volunteers who did the same.

With that, thank you and we will move on to our panels.

Dr. Avindra Nath: Thank you, Brian. Now we have two panels. The first one is the study participants panel that will be chaired by Vicky Whittemore, most of you know Vicky well. She is a neuroscientist and in the Office of the Director in the extramural program at NINDS, she is a Program Director and oversees the ME/CFS program. She has a broad understanding of this disease and she's going to chair the patient panel. Thank you.

Dr. Vicky Whittemore: Thank you, it is my pleasure to introduce this panel and to specifically thank all of the individuals who volunteered as participants in this study, and also to take this opportunity to thank all of you scientists who have been part of this study, it's amazing to hear all of your results presented here today. Thank you very much.

Today, you're going to hear from David Reimer and his sister Sue, from Saana Stella, and from Afton Michelle Hall, they recorded their messages for you and we will listen to their messages now. I'm not sure how we get them to play. Great, thank you.

Saana Stella: Hello, my name is Saana Stella and I'm one of the study participants. I hope to be able to provide a little bit more context for where the results came from. I became sick very quickly the day after Thanksgiving 2014 when I came down with what was diagnosed as bacterial bronchitis. With medications that I was given, they did their job, and I was feeling better in terms of the bronchitis. However, I surprisingly kept getting weaker and weaker and my body felt like lead, I could not move it. One month later I was unable to get from the sofa to the dining room table, therefore, became more sleepy over time.

Forward to 2017/2018, I was once again stuck in bed and got really frustrated and angry. I channel that energy into five clinical trials. It was the most taxing and hard thing, but I was thrilled when I was able to participate. The entire process of just even applying showed that they were very thorough and caring. I was ready to go and do whatever I could.

I was there for about two weeks total, two weeks the first time and in 2018 and another two weeks in 2019. I was able to get there by plane, using my wheelchair and had assistance from my husband and a friend. However, after three or four days of testing I was so crashed and was only able to get to testing on a stretcher. I was using a bed pan, I was unable to even do anything much. I wasn't even unable to sign the consent form, I think it was a while when someone noticed I could not lift my hand and write my own name at that point.

It was very, very tough, but in my mind, it was worth the effort, absolutely.

I also, they realized and I unfortunately, did as well that of course, the way I crash, even if I was able to sit on that exercise bed for the CPET for some time, I would absolutely be unable to be in the metabolic chamber for 24 hours without assistance. So, I therefore was not able to participate in the CPET part but they did invite me to do a modified protocol, so I was able to go for a second visit.

I always felt that the team listened, cared for me, and truly wanted to make progress for all of us. One example would be that when I arrived for the second study visit, there was a new rule that was supposed to be implemented throughout the entire Clinical Center where we had to basically demonstrate our functionality by marching in place, standing, showing stability in those kind of things. Twice a day.

For me, that would have been a huge effort is to demonstrate that I was failing a lot of these functional tests and I expressed my concerns to Dr. Brian Walitt, he immediately went to the highest levels of the NIH to request an exemption so that I wouldn’t have to spend my energy on showing that I can sit up, stand up, whatever it was.

I felt heard, I felt he was taking it seriously, I felt cared for and I was deeply grateful because, we all learn how to pace and make our own choices about what to spend our energy and effort on. I was determined to put everything into test results and not anything else.

So, then usually by the end of my visits, I was completely crashed and I would say most of the results they gathered, think there 279 results in my chart, most of those would be PEM or crash results because I was not in good shape. Pretty quickly.

Getting home was always difficult as well because I could not fly back, so my husband had to drive all the way from Chicago to Bethesda with an air mattress in the back of our van, I was wearing a diaper and I just, it was pretty unpleasant.

So, the – yes, I would say both the application process, during the study as well as after, the care team surpassed my expectations and they would check on how I was doing later on, and just showed the utmost care and thoughtfulness. I am very grateful for their work, and therefore, it was pretty difficult to process some of the criticism that I saw after the publication of the results because it was in such contrast to my personal experiences. I hope that this was helpful to some to see, hear from one of the participants on what condition they were in and they always felt that everyone on the team, especially Dr. Brian Walitt who was there doing a lot of the tests himself always felt accessible and wanting to take care of his participants as best as he possibly could.

I hope this leads to a lot more, and I thank you for listening.

Afton Hall: Hi, my name Afton and I'm pleased to be here to share the effort in the NIH study to solve ME/CFS. One of the most difficult decisions that I've ever made was determined to live a contributing life despite having ME/CFS. Prior to ME/CFS I was a busy person, it was not unusual for me during the workweek to go to work for 60 to 80 hours, I slept an average of 42 hours and the remaining hours were spent doing housework, contacting loved ones, various activities and helping others.

My mornings would start early, and I would get my husband off to work, and get in late after a full day, I loved my life. It was fulfilling. March 6, 2016, at 13:40 my life shifted, I felt my life receding and by 14:15 could not walk without assistance or hold my phone to call my husband, I could not speak above a slight whisper and I was compromised to the point of requiring help to shower, brush my hair and be fed, and needed help rolling over. I saw myself stop because if I was awake and could take a call, it lasted less than a minute. I had a circle of protection from the start that I never expected to need.

My sweet husband and my family became my advocates. My husband, children, daughters and son-in-law's, my grandchildren and extended family all rallied around. I was that compromised that a request that many people to help. I felt like I had a wall that was insurmountable. My one daughter fixed me a schedule to help me move, it reminded me that I had daily tasks. I have a daughter-in-law that would buy me any gadget she could think of receipt of position my phone and tablet so I could remain in contact with the family. Financial support was given as various treatments were tried as they were not paid for by insurance and are highly expensive.

Signs of crashes were observed and shared so everyone could help me learn to manage exertion. Still, I could not actively participate in my life for two years. When my practitioner found the NIH study and helped me apply, it a very arduous task to fill out the paperwork and there was the wait time as the process to verify whether I actually had ME/CFS. It was a blessing to be accepted and it gave me a renewed sense of usefulness in spite of severe limitations.

Upon learning I had been accepted into the study, my husband and I begin a series of purgatory test to see if I can withstand the trial. My husband built a bed for me in advance that he specifically bought so he could be able to tote me around. We took one trip that was 1200 miles long so we can find the perfect amount of time to drive and still allow me to move the next day. We found that sitting at a 30-degree angle for five hours worked if I was upright, I could travel for an hour and a half. This may sound trivial, however it was necessary to do the math so that we could carefully design the best way to get me across the country without causing so much harm that I could not participate once I arrived at the NIH campus.

The NIH provided me with the schedule before I arrived it was overwhelming, it felt like I was going to be a guinea pig. At the time, my sleep patterns were accumulative of 18 to 22 hours of sleep a day. The longest I had been staying awake was one hour. The schedule clearly was going to require more than that. I could stay awake and upright for about 15 to 22 minutes without any adverse effects, literally at 25

minutes I start to shake, fall forward, my speech would be slurred, feelings of immense shooting pain, a symptom I refer to as the electricals. They would begin and I would be unable to focus on conversations.

Light and sound became to be unbearable, and I would have to lay down and try another day. Life is all about that, and everything has a pattern. I decided if I can participate in developing the bank of information and data that data-driven science it would have a new deposit. It's difficult to adequately describe my experience with the NIH, most of my time was spent trying to stay upright. I can say is, I don't know 100 tests and surveys were, it may have been more or less, but it certainly felt eternal. I never experienced such good care as I did from the staff at NIH. Starting with the very first contact from Angelique and Dr. Nath, and Dr. Walitt, as most recently as a few month ago, my trust in the entire team of strangers was validated and I owed them my determination to continue to live with my best effort.

My sweet husband and one of my nieces, two of my daughters and my son-in-law all make sure that I have support there at the NIH from family also, they've came from as far as Utah, New York, and Washington, D.C. to make sure that I still felt connected and that my work was of value. Barbara Robertson I believe was her name was an occupational health therapist and she helped to make sure I had Social Security Administration benefits to the maximum possible.

She then talked to me about my sorrows about my lost occupation and ability to cook for my family. I had not shared those sorrows with anyone prior to that. She knew what questions to ask and she helped me face those and to come up with a solution. She kindly made sure I had a tilt chair sent so that I could cook for my family occasionally. When I say occasionally, I mean three times in one year. It helped me out and shows how grateful I am to this day that I was able to those three times.

There was another test that occurred that was particularly distressing and it was an electromagnetic testing and the scientists there were so very concerned about my comfort that even more so, my team with Dr. Walitt who I interacted with daily sent Joy so she could be there for the entire time, and make sure that they did not cause any harm. Everyone was so very careful to listen.

Another time that things were very protective was when I was having my spinal tap. The second one, and they were very uncomfortable. Dr. Brian Walitt said if this becomes too much, just say stop and we will stop. So, I weakly said "stop" at the point where they needed two CCs more for a little extra test, and I weakly said "stop" and the scientist did not hear me. Dr. Walitt was there, and he said, "she said stop!" and the whole room stopped. That level of protection is invaluable. Since reading the synopsis of the results and attempting to read the full study with my current limitations I feel relief, I personally am grateful to know now that my body has had a system disruption.

I know that scientists can now move forward with additional information and I can help them in their studies. I willingly completed the study, and I accepted the impact on my ability to function. It took nearly eight months to return to my level of functionality prior to participating in the study and it's been a difficult journey to have an illness that is having terminology developed and defined. Developing a databank of information that is verified can be replicated is undeniably critical. The ME/CFS patients need to have answers as soon as possible. I'm grateful to have been a participant in such an important body of work. I, a patient with ME/CFS, now have hope that my children's generation will benefit from our sacrifices by seeing acceptance and acknowledgment in the public and by the CDC, the medical community, and the rest of the scientific bodies that are working on this.

I hope that my grandchildren's generation will have interventions to manage or cure this debilitating illness, and am truly fortunate both will occur in my lifetime.

Again, I, a patient, would like to thank you for this opportunity. To hopefully give you the scientists that we depend upon a sense of my gratitude for your willingness to research and refine the information, to seek out the causes and solutions for ME/CFS, it's a very complex, misunderstood, and underfunded disease and I appreciate your sacrifice. Thank you.

David Reimer: Hello, my name is David Reimer. Prior to ME/CFS, I was a family physician with training in tropical medicine. I worked in inner-city Toronto, and then in the Himalayan country for five years before settling into a small town in general practice in Canada. As well as medicine, I was involved in hospital and nursing home administration, teaching, and several community groups.

I made sure to set aside plenty of time for my wife and three children. Throughout my life, I had enjoyed challenging myself in many outdoor activities including wilderness canoeing, sailing, hiking, downhill skiing, long distance cross-country skiing, and running.

It was on a family trip in 2016 that I picked up the virus that resulted in my post-viral ME/CFS. Step-by-step over the next three and a half years, I lost the ability to do all of these things. When it was clear that I had this poorly understood condition, I looked for research studies investigating ME/CFS that would contribute to better understanding of it and maybe even of help personally.

So, it was very encouraging to find this NIH study in progress with such high-quality researchers and extensive resources trying to figure out what was going on.

I was impressed by how very thorough the intake process was and ruling out any other possible causes for my symptoms. It was fascinating being at the NIH, and part of so much high-tech testing such as the metabolic chamber, plasmapheresis, genetic sequencing, tilt table testing, and the transcranial magnetic stimulation. Positioning their magic wand over the precise place on my head to stimulate a single muscle on my right forearm.

Throughout the study, the team was very supportive and accommodating of my needs for quietness, rest breaks, and other assistance. Overall, participating in the study was very affirming and encouraging, and definitely interesting. Thank you so very much to all the research team, I'm grateful to have been able to participate.

At my lowest, in the year following the study, I was approximately 15/100 on the disability rating scale. I've improved significantly since then, but by this time in the afternoon, I will be sleeping and not able to take part in the discussion. I do have a very supportive family. So much so that one of my sisters, Susan, also took part in the study as a healthy control and she has her own story.

Susan: Hi, I am Dave's sister, Susan. I participated in the study as a healthy volunteer. I saw my brother suffering in such a profound way from the strange and mysterious illness, I signed up in the hopes of learning more about the condition than doing my part to help find a cure for my brother.

Participating in the study was a fascinating experience and they had me busy from dawn until dusk for a week. It was slightly intimidating to be monitored by such brilliant minds while I did math equations in the fMRI machine. During the muscle biopsy, we all gave a thumbs-up and I sent this photo to my brother with the caption “Team Dave.” Dr. Walitt was closely monitoring for variations and test results between my brother and I.

Participating in the study was transformative and inspired me to take my health more seriously. I took up running and was training for a half marathon to mark my 60th birthday, I went backpacking in the Canadian Rockies. After the lockdown, I contracted a serious case of COVID despite being fully vaccinated. I never thought it would happen to me.

I’ve always recovered predictably from illness, so I started gentle exercise, but despite my best efforts to rest and pace, I became progressively worse. My body was completely dysregulated and soon after I was diagnosed with Long COVID. At this point, I reached out to Dr. Walitt, given how thoroughly I was tested when I was healthy, I felt that if they tested me again, it would result in valuable data. I came back to NIH and participated in a natural history of post-coronavirus study, which in many ways can be seen as a sequel to the ME/CFS study.

Participating in a more recent Long COVID study has been such a blessing in my life as it broke down some of the loneliness and give me an opportunity to bring some good out of my own story. Thank you from the bottom of my heart to Drs. Walitt, Nath, and the NIH staff for your wisdom, compassion, and sound guidance. I'm very aware that my story is part of much bigger story, and I'm grateful to play a small part. Thank you.

Dr. Vicky Whittemore: Thank you so much for your contributions. Are the individuals on Zoom? There they are, welcome. So, any questions from anyone in the audience? For these participants, any comments?

Dr. Avindra Nath: I would start by first of all, thanking each one of them. Not only for participating in the study, but also for sharing their stories, and just making these videos is not an easy task, especially when you're suffering from this disease, sharing your own illness with a broad audience, it requires a lot of courage. These are the patients that really make, the movers and shakers, and really make things happen that wouldn't happen otherwise. For that we are extremely grateful.

We have other patients here in the audience that have identified themselves previously. I welcome them to come over and maybe comment if you want, on your own experience, and how it may relate to theirs. We learn from you and that is what we use for the purpose of research, so here is a great opportunity for you to do that.

Joe Lanson: Hi, Joe again, a patient. Everyone worked very hard in the study, but having experienced post-exertional malaise off and on for 19 and a half years, I honestly do not consider it to be a subjective experience. It would be the last way that I would describe it. Thank you.

Dr. Cheryl Lohman: I have spoken before and I will just say, my own personal experience is similar to yours. Thank you.

Dr. Vicky Whittemore: Sue and Saana, do you have anything you would like to comment on before we wrap up the panel?

Susan: I would like to thank everyone again. It's been a transformative experience, and I'm so grateful to the entire team. Thank you.

Saana: I guess I will also agree that absolutely, gratitude is the most prevalent feeling and also, hope and excitement that this is just the beginning. It will lead to much, much more to come. Thank you, everyone.

Dr. Vicky Whittemore: Thank you very much, thank you everyone.

Dr. Avindra Nath: Now we have an esteemed panel of scientific experts here, and I would like to welcome them to come over here in front. We have two people who will be online, so it will be a hybrid panel and we will see how we can make that work.

So, we have experts from a wide variety of experiences with, researching with ME/CFS. I want to introduce them to you, Vicky Whittemore as mentioned already, is the Program Director at NINDS and she oversees the ME/CFS research program as instrumental in putting together the Roadmap for ME/CFS. So, that ensures that the research will continue for as long as we can, is necessary to cure this disease.

Joe Breen is a Program Officer at NIAID and Chief of the section on immune regulation and has been a pioneer in funding this research, overseeing the centers, and has been a very strong supporter of ME/CFS research.

Together, they oversee the entire portfolio across the country funded through NIH, so they have a great depth and breadth of knowledge about the disease.

Next, I wanted to introduce Dr. Anthony Komaroff, and he is online. He is the distinguished Professor of Medicine at Harvard Medical School, Senior Physician at the Brigham. He's had many leadership positions and is really a pioneer in ME/CFS research and has been a very, very strong advocate of this research and the disease, and for the patients themselves. We have learned a tremendous amount from him, he was on our adjudication panel and when we first started the study, he gave us excellent advice on how to really shape it and what to do.

Throughout this process, he has continued to be a champion for us. We thank him for that and thank him for taking the time to serve on this panel. I know he would have liked to be here in person, but he had a pretty long trip from the west coast. He was unable to do that.

Next, I would like to introduce Nancy Klimas who is also on Zoom. She also has over 40 years of experience with ME/CFS. With research and taking care of these patients, and we have learned a tremendous amount from her as well. She also is instrumental in referring some of the patients to us, she's the Director of the Institute of Neuroimmune Medicine at Nova Southeastern University. She is also the Director of the Clinical Immune Research Program at the VA hospital in Miami and Chair of the Department of Clinical Immunology. Thank you very much for serving on this panel.

I would also like to introduce a very close collaborator, friend, and expert, Dr. Ian Lipkin. He's the John Snow Professor of epidemiology and also the Professor of neurology and pathology, at Columbia University. He is also the Director for the Center of Infection and Immunity. Most people know him as the virus hunter – he's discovered more viruses than anybody else on this planet, from West Nile, to MERS and SARS, and has been involved in the SARS-CoV-2 pandemic as well.

He's been very strongly committed to really studying ME/CFS, and to really devoting the rest of his academic career to this disease and he's been a very strong advocate for treatment trials of this disease. We have learned tremendously from him and even throughout the day

today, he was trying to tell me how to best to do the various kinds of analyses, which we talked through, which I'm greatly appreciative. Thank you for serving on this panel.

I will turn it over to you now to discuss the findings and where we should go and how do you want to handle this? Dr. Komaroff, let's see if you can moderate for us?

Dr. Anthony Komaroff: Yes, I'm happy to do it. Thank you so much for inviting me to participate, I'm sorry the commitment on the West Coast made it impossible for me to be there today. I wanted to just start with a few short comments to put some context on today for other people to respond to.

About 40 years ago with interest in ME/CFS resurfaced, we really didn't know, hadn't identified any underlying abnormalities that could explain the symptoms, and that led some people would say, there's nothing really wrong with people with this illness.

Over 40 years, however, there are now about 10,000 peer-reviewed publications that involve thousands of patients with this illness that have revealed, replicated underlying biological abnormalities of the brain, autonomic nervous system, immune system, energy metabolism, gut microbiome, cardiovascular system. And the study being presented today is the most intensive study of with people with ME/CFS that has ever been done. More measurements of more body systems than has ever been done before. That is very important, it has confirmed the results of many past studies and it's also uncovered, as we heard today, some new abnormalities that have not been previously reported.

This is, as I understand it, if you wanted to boil down what this very complicated study with massive amounts of data has said – it says that ME/CFS is a brain disease likely induced by chronic immune activation and exhaustion. One novel finding that was described a few hours ago is this involuntary underlying abnormality in one part of the brain that involves a deficiency of the brain chemical norepinephrine.

That abnormality and the immune exhaustion found by the study have both been identified by the study as targets for potential treatments, and they also open the door to studying the underlying biology of the illness. I think this is all really good news that adds to the growing recognition of ME/CFS, Long COVID, and other post-acute infection syndromes. I might say parenthetically that there is one measure that recognition today, paradoxically, Time Magazine announced its list of the 100 most influential people in global health. This covers all diseases, people all over the world and of these 100, one is a patient with ME/CFS, she is a patient activist, and three are scientists working on these illnesses, including the senior leader of this study, Dr. Nath.

I think that is congratulations to them, and good news that this illness is being recognized more, and more, and studied more. With that, let me throw it out to the panel for any observations today.

Dr. Ian Lipkin: I've had the pleasure working with Tony for more years than we would like to count. He's very good at summing up the state of knowledge and so forth. I want to echo his comments about the study. I think there is no place other than NIH where this could have been done, and I think the leadership has been extraordinary. Is this the last word on ME/CFS? Obviously not.

What I wanted to do was try to summarize what I took from today's conversation. To discuss, perhaps, the implications of some of the findings.

The immunology of ME/CFS goes back a very long time. I can tell you that we found evidence of abnormalities in B cells back in the mid-1990s, many people have reported elevated levels of cytokines, changes in cell counts, today we heard about this molecule called PD-1, associated with immune exhaustion. This is a molecule that is targeted in people of certain types of cancers like melanoma with antibodies who you can suppress its activity. The importance of this covers several different areas. One of them, of course, is if there is a persistent infection, and I'm not saying that there is, I'm saying if there were, increased levels of PD-1 or any of the associated molecules would result in difficulties in clearing infections. That again, brings us back to, I'm afraid, it's a little chronological because this particular study was focused on patients who had infection.

I think it represents the majority of people with ME/CFS. Now, what do we know about infections in ME/CFS? Because we have been talking about that for a very long time. Our center has been trying to find bacteria, viruses, fungi, linking them to ME/CFS for now many, many years

using very, very sensitive methods. We have not found that, we have not found any such evidence except for the microbiome work, which I will talk a little about in the next section.

That doesn't mean that infections aren't implicated, it simply means that either we are not looking at the right, in the right place, or at the right time, or at the right sample, or that there may have been a hit-and-run mechanism, which I frankly favor. So that you're not going to find it using direct detection methods, you have to use other approaches looking for footprints of exposure, and this brings us to antibodies.

The challenges with antibodies is that there is no huge test that allows you to simultaneously test in a very rigorous way for exposure. There are various methods that are coming online that will allow you to do this to some extent. I think this is going to be extremely important. The other issue then gets back to timing. So, if I do what we call a cross-sectional analysis, if I look at one point in time and maybe I look a year later, I may have missed some sort of an event which triggered disease that is important.

There are two approaches that we have taken, and I'm referring to work actually that Tony and I are doing together, where we look at individuals who had an exacerbation in their symptoms. We then go in and specifically look to see if we can find evidence of infection. At that time point, and two to three weeks later, we go back to look for evidence that antibody profiles have changed. The other approach is to use a repository at the Department of Defense that was successfully used to look at triggers for multiple sclerosis a few years ago, and there you can look for people subsequently developed ME/CFS or a related syndrome and ask whether or not then the year prior, because these samples were collected annually, whether or not there's been an exposure that might be linked. We're just going to have to stay tuned for that because we don't have those data yet.

The other aspect of the immune system I think it's worth talking about, which some people alluded to, which was the innate immune system. The innate immune system is very primitive, you can find it in fruit flies, worms, and it's the oldest sort of protection that animate beings have for eliminating viruses, bacteria, and fungi. If it goes awry, if it’s not somehow controlled, you can wind up with the expression of various molecules that gives rise to sickness behaviors for example. They can also have indirect and direct effects, as well as viruses do, on mitochondria and impact that function. This is something, again, that we need to look at.

There was a discussion about microbiome. I think it was very elegant conversation about the microbiome. Butyrate was described earlier as being a very important molecule, it modulates immune responses, it also maintains the integrity of the colonic epithelium. It keeps what is supposed to be outside of the body outside and prevents it from going inside. The implications of these first things that we had talked about are if we can find ways to dampen the immune responses, particularly the innate immune responses, something we should be considering with clinical trials. It has to be done safely and it has to be done rigorously, but it is something that the time has come.

Similarly with butyrate, the microbiome, there are ways by using prebiotics, which is specific foods that encourage the growth of specific bacteria, and probiotics that actually change the composition of the gut. These are things that we can also be pursuing, and I think again, the time has come to start thinking about how to do this. I keep coming back, as I think about this disease – excuse me, about the metabolic factors.

When the mitochondria is dysfunctional, when anything is dysfunctional, there’s a variety of very small molecules that are released that you can measure the quantitative profile. The problem of course is if we are really interested in something that's happening inside of a cell, it may, that particular factor may be diluted by the time it gets out into a fluid like plasma where we can measure it. Nonetheless, despite this lack of sensitivity, we and others are finding signals I think that are going to be very, very important.

What we didn't talk a lot about today were sex specific differences. I think that this is an oversight, possibly because there weren't enough subjects in the sample. In our experience, more women are affected than men, and women of different ages have different sorts of metabolic abnormalities and we have found problems with certain cycles in the mitochondria that result in the elaboration of the energy molecules, the ATP that you heard a lot about earlier. There are also lipid abnormalities linked to problems in the mitochondria that are associated with inflammation.

Some of you may remember this movie several years ago called "Supersize Me" has anybody seen that? This guy goes on this diet, goes to McDonald's and every time he goes, when they say, “supersize me” he eats whatever they give him. A large french fry portion, coke, whatever it is, and he winds up developing this inflammatory disease that is associated with this.

There is a very rigorous dietary diary that was kept, so we know that that is not the case. I'm simply trying to say that lipids aren't necessarily good for you. This is something else that we need to explore. The neurophysiology portion of the talks, I found probably the most interesting because I'm a neurologist and we like to look at pictures of brains and how they function and so forth. I was somewhat surprised by the tilt test findings. I will admit that, because so many of us for such a long time had been hearing from patients about problems when they stand up, they feel like their blood pressure drops and so forth.

I'm very sensitive to this because I have another problem that gives me the same sort of symptoms. We didn't really see that, and I don't understand why that is the case. I'm not saying it is not true, I'm just saying it was surprising. Cindy Bateman has been doing this lean test for many years and has found a variety of abnormalities in pulse pressure and so forth.

The findings and catecholamines were really interesting, and I don't know, I'm wondering whether or not everything was associated with a decrease in synthesis or whether or not there might be problems in reuptake? And I don't know if Goldstein is still here? There he is. That was another question, that I wanted to ask because if so, maybe this would be an indication for using things like reuptake inhibitors. Those are really my thoughts, I was also surprised that there was nothing in the nucleus accumbens, but those are the data.

That is all I have to say, except that to the patients, people with ME/CFS, people with Long COVID, you are heard. We don't have enough resources to do what we need to do, and if I have learned anything after 40 years of doing this kind of work, you need better advocates. The squeaky wheel gets the grease. Over.

Dr. Anthony Komaroff: Can I ask Vicky Whittemore to comment on today's presentation?

Dr. Vicky Whittemore: Sure, thank you Tony and thank you Ian, that was really a very nice summary. So, I'm looking at the data that I heard from when I first heard the words chronic fatigue syndrome, when I joined NINDS and asked if I would take two small grants on autonomic dysfunction on ME/CFS, because my previous work had been on autonomic regulation in animal models.

I said sure, but I knew nothing about the disease. I thought it was just people being very tired. I have learned a whole lot about the disease since then, and I think my window into the research has really changed since 2015 when Dr. Koroshetz agreed to take over the Trans-NIH ME/CFS Working Group and moved that to NINDS, and Joe and I have been working closely with him since then.

Just looking back at the data and the research that was being done, there was, and where we are today, we need to consider the length of illness, we need to consider the sex of the individual with ME/CFS, we need to consider what is potentially the trigger of the disease. There are a lot of factors that if you look at the older literature, everyone was put into one bucket – whether you had been ill for three years or 30 years. We are finding from the results from the ME/CFS Collaborative Centers that there is a significant difference between the individuals who have been ill for a few years versus those that have been ill for ten or more years.

I think all of those factors are critically important as we think about the disease, as we think about performing rigorous research on the disease. As you heard, Dr. Koroshetz, mentioned the Research Roadmap that we have been working on now for a year and a half, I encourage you to tune in on the afternoon of May 15th when the report will be presented to the NINDS Advisory Council. And the report is really just the beginning of continuing to really move forward and stimulate research.

I'm not going to steal the thunder of our presentation on the 15th, but only to say that we went into developing that report with the whole premise asking everyone who participated, all the speakers who participated in each of the eight webinars that we held: what do we know, what don't we know, what do we need to know to move to clinical trials? To really put the focus on where is the scientific evidence where we can, as rapidly as possible, get clinical trials going for the individuals with ME/CFS who have been waiting for so long.

Clearly, they need, as Ian said, they need to be done safely, rigorously, and thoughtfully, but that is the future direction of where this Research Roadmap will hopefully take us to really accelerate the research into new treatments for individuals with ME/CFS.

I think the, all the data I heard today, I think again, as has been said, replicates some data that has already been presented, there is some data I know about there that is replicating, would you have found, and I think also, opens up avenues for a lot of extensive research. When you see the research report, you're going to see that we had significant difficulty narrowing down the research priorities. There are probably

more than 100 research priorities in the Research Roadmap because that just indicates how much is yet to be known and how much is yet to be done to really move towards treatments.

I think the research that has been done here at NIH has really been significant, and really helped to, again, move the field forward in ways that couldn't have been done in studies that couldn't have been done anywhere other than here at the Clinical Center. Thank you.

Dr. Anthony Komaroff: Thank you so much, Vicky. Joe Breen, any thoughts?

Dr. Joe Breen: Just a few thoughts. So, NIAID has had a long-standing interest in ME/CFS because of this connection with post-infection that we have never completely been able to identify an agent. The connection is there, epidemiological data is clear, that it looks like a virus but we have never been able to isolate whatever it is.

So, I have a few thoughts from today, I guess we just heard some from some patients, and I was really struck by their contributions, of course to the study, but the idea that the hope that grandkids would benefit from this program really struck me because I hope, I really hope we don't have to wait that long. That really hit me kind of hard, so I wanted to note that. That showed a lot of grace, frankly.

I come to this, but like you Vicky, we started this effort 10 or 15 years ago when we reorganized the NIH and how this disease would be approached. My experience had been in post-Lyme disease prior to that. I'm also struck by the commonalities in post-Lyme disease, other chronic post-infectious sequelae, and of course, Long COVID now. Like it was mentioned earlier today, I do really expect that some of the benefits from learning about Long COVID are going to translate to ME/CFS. If nothing else, the structure on how to study it with outcomes that are patient derived and standardized across different mechanisms, and interventions. I don't know if ME/CFS was identified prior to COVID as the same as Long COVID, I don't think there is, we know there is overlap from what we can visualize.

Perhaps when we have the results of the more in-depth phenotyping that Dr. Nath are others are leading here, we will have that, but we don't have that evidence yet.

I'm hopeful today, because I saw new avenues of research and understanding that should hopefully lead to completely different interventions that I would have guessed from an exhausted T cell phenotype, for example, or the PD-1 one pathway, utilizing some of the neurology that we saw today. We saw clues from the December ME/CFS meeting about brain imaging, then we saw some fMRI in the Nature paper. I think those are all areas that now can be thought about by a number of groups and explored, hopefully.

I'm enthusiastic about that, but tempered by the talk from the patient, that today they feel terrible, and these things take a long time to develop. I look forward to the challenge, but it's a healthy respect of the suffering that is occurring at the same time.

Dr. Anthony Komaroff: Thank you so much, Joe. Finally, Professor Nancy Klimas, can you tell us your thoughts?

Dr. Nancy Klimas: Sure, I'm trying to remember Tony, the first time you and I talked about this was in the early 90s, right?

Dr. Anthony Komaroff: I think it was the late 80s.

Dr. Nancy Klimas: It may have been, I remember that. I was just thinking, some of the things discovered in this effort, of course, were in those early papers as well, the chronic immune activation and the, many of the inflammatory markers and so on. What the study has done, and I love to look at that evolution over time, has been a lot more comprehensive study when it comes up with some of the things you expected as well as then you can believe the things that are unexpected. If the study showed immune exhaustion, I think others have been showing immune exhaustion and more recent ways of measuring that.

The low stroke volume, that is such an important clinical observation that we act on every single day in our clinical care, and I think others have also remarked on these type of things. The bioenergetics observation is critically important to know. Not just if the cells are broken, energetically speaking, but when did they get that way? Is this a consequence of long-duration illness or is it something that happened early on inside of the illness? These are really important cutting-edge questions.

What is special about this particular study, and I like to think of it as the, that old elephant in the blindfolded wise people hit on the trunk and feel the legs and say no it's an immune disease, no it's a viral disease, no it's a bioenergetics disease, no it's microbiome. The point of this is you have the whole elephant in the study, and that is what is incredibly special about the study is the depth of the work that was done in the study and every single aspect that every way you can peer through the window to look at this illness, they peered through and looked at this illness.

This first paper sort of broke up the pieces to demonstrate what the view was, but the real strength that will come from this, and I'm excited about this because our group will be helping with this, is taking all that data and modeling it into mechanisms of illness and therapeutic intervention, targets for therapeutic intervention. Then the whole bit that Brian talked about right before the panels about data sharing and open access to data, that is the most important, it's great you did this work, but how amazing that we all can now dig into the dataset and do it in a way that could potentially teach us new things.

Our group has been doing dynamic modeling and coming up with models and interventions for a number of years now. We looked at it through the immune window, we looked at it through the neuropeptides, and we looked at it through cellular function, we even looked at it through what is the brain doing, yet the brain is the most important part of this.

So how cool is it with this modeling work, that we will be able to take on this neurophysiology data and drop it into the modeling systems and make it, the science word is granular, the most granular dataset you can get out of the most data that you can pull into the model?

One of the things that is exciting in working with computational biologists is that they are never capped by too much data, they think more is better. I love that. When you're dealing with five, six, 20 billion database points per time point, and they're like no that's great, more would be better.

That is very exciting. The other thing is, comparing this ME/CFS to these other illnesses that had acute brain triggers like Long COVID or in the case of the study that the VA is proceeding to do in partnership with Dr. Walitt and Dr. Nath now in Gulf War illness, which is a neurotoxic acute injury in 1991 that has, well one veteran in three that was deployed there is still ill today from their exposure to neurotoxins in 1991. That's a really important group who presents identical to ME/CFS, I saw them on in the same clinic and couldn't tell one from the other, I just asked about how things got started, and yet biologically there are differences and similarities.

The science question is, the differences matter, that is important in my influence of treatment. The similarities really matter, what is the commonality between these illnesses that has a clinician like me scratching my head when we walk in the door and I didn't know the history, I couldn't tell Long COVID from ME/CFS from Gulf War illness or another mold exposure injury or other type of toxic injury. Because they do present in very similar ways. I think what we learn using what we now call AI but have been calling computational modeling for a long time is going to give us insights that are translatable to intervention.

So, Tony and I could sit here and Ian as well, we have been taking care of these patients for a very, very long time, we have been wanting to move this needle and get onto clinical trials and give them focused therapies that are more than symptom management, and you need this kind of science to push that through.

If I were to underscore what happens next, it is let's get to clinical trials. Let's take this type of data, spin the hypotheses that it will generate, do the next level of studies, but at the same time, take the things that were validated by this study and others and act on those information. It's not that we don't know a lot about what is wrong, and that we wouldn't have reasonable hypotheses that involve a clinical trial to test to get people back towards health.

I'm just going to say it was a great study, an important study, and one other thing I heard people complaining about a lot, and yes, it would be great to have more, but you can do n of one modeling and we do that. We can get down to a single individual and model the disease in that one person. That is very cool. If you can do the modeling with this big group analysis, break the men and women apart, or break long and short duration apart, but you can also just take one at a time and model each individual person's underpinning of illness and then find where they overlap, who hangs together, and that is a whole other scientific approach which our group is definitely engaging in and this type of highly granular data set will allow that.

Dr. Anthony Komaroff: Thank you so much, Nancy. Speaking of next steps, I think that is the next panel in this meeting.

Dr. Ian Lipkin: Tony, I want to say a couple of things in response. We have talked about early versus late disease, this is extremely important. When you look at cytokines early in the disease, they tend to be up, you get to a point where they – the people appear to burnout and the levels drop. As you're asking different patient populations, you have to make sure that you are comparing people with recent onset versus like Vicky was talking about, ten years later. If you mix those two populations, you may miss signal.

The other thing is, about the therapeutic response, the time course of this is not going to be short. As we develop clinical trials, it's important to make sure that the drug, whatever it is, whatever the intervention is, continues for months because it took months to generate the problem, and is not going to be a simple fix.

The other thing is, even those clinical trials where we think it is unlikely that it's going to pan out and result in identification, a panacea, if we learned anything from XMRV, MMR, autism and so forth, it's very important to test those things that even if they yield some sort of negative result, will result downstream in people shifting away from things that are inappropriate and allow us to focus on things that might be constructive. Thank you.

Dr. Anthony Komaroff: Thanks. Thank you to all of the panelists, I will turn it back to Dr. Nath for a summary and future directions.

Dr. Avindra Nath: Thank you very much, that was really outstanding and thank you for your support of our studies and for your advice now and throughout the study.

I just have a couple of slides here, just to summarize some key features and so, in summary what I'd like to say is that ME/CFS, as you heard, may have underlying treatable diseases. As you know that some of these patients who were brought over here and we studied them extensively, we found that there were other diseases that were underlying and were missed.

So, I think it is very important that as a physician, that when you see these patients, that they be closely followed, it's impossible for everybody to, all physicians to take these patients and do a million-dollar workup on them, that's not going to happen. But if you follow them along you'll eventually discover if there's an underlying disease.

The second thing is that we learned that some patients will spontaneously recover. So, that again is very fascinating, for us to be able to identify who those individuals are and why that happens can actually teach us a lot about the disease and how we want to design our clinical trials. That's another thing, I think that as physicians, we need to pay attention to.

The third thing is that multiple biological systems are involved. We focused on the brain, that is where our expertise is, but what you have seen through all these presentations and extensive work that we have done, is that the nervous system does play an important critical role, but it doesn't mean that other systems are not involved. We need to pay more attention to them and maybe study them more extensively.

Then there are multiple targets for intervention. Several people have mentioned that, and I think that is an important outcome of this study. And that there are key sex differences in the pathophysiology of the disease, which means if you're going to develop treatments, we need to pay attention as to who we are treating and select the right population for treating them. One size may not fit all.

So, then what about therapeutics? Going back to the figure that you have seen before in many different ways, I want you to know so you that when you think about therapeutics, you can divide them into two parts. The first is, is it possible that if you attack the early stages, which is the immune system itself, could you actually reverse the disease, halt it, or maybe slow down the progression? I guess that is one possibility.

The other thing is maybe you could still intervene over here, and actually cause symptomatic treatment, and intervene, and really help the patient in many different ways.

The thing is that if you were to be able to, the question is that if you were to be able to act here, can you prevent everything downstream? I don't know if that's possible or not. If it's reactionary, it certainly might be possible. It might be possible if you catch your patients early on, you're able to make an intervention here and provide all these things downstream. The other possibility is that this part of the figure here

could become self-perpetuating, and that happens most commonly with pain syndromes. What triggers the pain becomes the self-perpetuating phenomenon, so you can treat the underlying cause and the pain persists.

If that is the case, then you need both. This alone may not be sufficient, that doesn't mean if you see immune abnormalities try to fix them, but at the same time you're going to need this as well. Going to need combination therapies in order to be able to handle the process.

I will talk a little bit about this early part of the equation here. If you think there is an immune mediated phenomenon, what choices do we have?

So, the other thing is, we should study ME/CFS but we can also be studying Long COVID patients. Now the problem is that a number of these patients also have had vaccines and have had COVID, so it becomes really difficult to sort out what is doing what. There's an opportunity to really do clinical trials in Long COVID patients and I think if we were to solve one, we would probably solve them all. I think it is really important that we select the right population to study. What would we do with this population? I think what we need to do is look and see what categories these patients fall into.

One of the things that I am keen to study is the population that has immune exhaustion, and if you were to use checkpoint inhibitors, can we really make a difference to the disease? There are individuals who have B cell activation, patients who have autoantibodies and such, we certainly excluded those from our study. There is an opportunity there to be able to intervene with the individuals who has the B cell activation in a variety of different agents that are available to us. Same thing with T cell activation, and as Dr. Lipkin mentioned, innate immune activation blockers as well. There is a number of different cytokine blockers as well.

Then there’s nonspecific immune modulators such as intravenous immunoglobulin, or immunoadsorption, and we currently have an ongoing clinical trial which is a placebo-controlled study, using intravenous immunoglobulin in Long COVID patients.

What I want to also emphasize is that we shouldn't helter skelter start treating patients with any of these agents because we will end up doing more harm than good. These agents are not innocuous, they have pretty profound side effect profiles. What I would urge that we do these things only in the context of clinical trials, and that is why I underlined and bolded this at the same time, it should be part of the clinical trial.

In the course of the study, the number of patients who keep emailing me with all kinds of things that they think we should be trying, here is a small list of things that they mentioned to me that are also potential immune modulators. Poly-IC, low dose naltrexone, thymosin alpha-1, Abilify, and various kinds of supplements. I think we need to look at these agents as well to try to see what makes the best sense, which ones to try.

The problem is now that if you were to now study one compound after another, I think we would be here for, it would take us a century or longer to be able to go through this list. Our lifespans are short and mine is shorter than a lot of you, so what we need to do is come up with other innovative ways of being able to do trials, and one way is to do what is called platforms. A platform study is one in which you can study multiple drugs simultaneously and compare to a single placebo arm.

Now, let's say you have five arms in that study, and you have one placebo, that means a patient's chance of getting placebo becomes 1 out of 6. That is an advantage to them, and you can compare and contrast these multiple drugs all at the same time. That is impossible to do in a single site, so you need multiple sites in order to do these kinds of studies, but that is one way to be able to get a quicker answer. Another way is to do crossover studies, that means patients put on a single drug or multiple drugs and multiple arms, but then they have an opportunity after they finish one arm to move on to the next arm. So that way every person who was a placebo has an opportunity to go on one of the other arms. There are these crossover designs that also can be very informative.

But then there are other future directions that we need to pursue, that is to continue the analysis and publication of data and samples collected, and Brian did a fabulous job of outlining some of the future directions we are thinking of. Reanalysis of data by other researchers, validation of findings in other cohorts, and as I said, we need to discuss and develop plans for clinical trials. We need to continue to study the pathophysiology in the context of clinical trials and I think that's important because you can never do enough pathophysiology, there is

always another question that remains unanswered and always another question. If we keep waiting until we have all the answers, we never really will get to the trials.

I think in the context of clinical trials, we can always continue the study pathophysiology. What I would advocate is that we hold hands together, move forward, with the patient community, with advocates and the researchers, and I think there is light at the end of the tunnel and I'm hopeful we will find cures for this disease. I will end here and if Dr. Schor is there online, I will ask her to give the closing remarks.

Dr. Nina Schor is the current Deputy Director of the Intramural Program at the National Institutes of Health, she is a pediatric neurologist who used to be the Chair at Pittsburgh, then the Director of pediatrics at the University of Rochester, and then she came to NIH as the Deputy Director of NINDS. It was during her rein that this study started, and she's been strongly supportive of our efforts at the NINDS. She was not only the Deputy Director, but she was also the acting scientific director so she played a very critical role in the intramural program at every single level.

So, I will give the podium to her for making some closing remarks. She is currently in Rochester and has a very busy schedule, but she made sure she would be available for this. Thank you very much.

Dr. Nina Schor: Thank you, Dr. Nath. Thank you to everyone who made it possible for me to participate virtually in this, and to watch the conference on videocast as well. Really, a privilege to be with you in whatever fashion.

I really want to underscore that all of the complexities that you have heard about today with regard to this disorder, disease, syndrome, all of these complexities are what make it so that this is a team approach kind of disorder, this is not a disease that we are going to solve or treat, or cure or prevent with an isolated scientist or physician in a laboratory or clinic. You have heard today that this is a disorder that involves the immune system, that involves inflammation at every level, that can involve the microbiome and changes that occurred to the microbiome, that can involve the autonomic nervous system, and injury to the autonomic nervous system, and because of that, it is a disease that takes many, many people approaching it from many, many different vantage points. It's also not a disease that is going to be solved by scientists and physicians alone, the partnership with our colleagues who are patients, who are advocates in this community, who are university, and government, and industry is critically important for us to be able to do what we do for this disorder.

I'm so grateful to all of you who have come together today in this context, and who have participated in these studies that you heard about today, and what we hope to do in the future, because without all of you, without the team, and without partnership, there is no way we will solve the kinds of disorders that are left for us to solve. It's in this spirit of gratitude, in the spirit of hope, and of partnership, and colleagues that I thank all of you for being a part of this very, very important conference today, for educating and partnering with each other, for educating me, and for allowing scientists like Brian, Avi, and clinicians like Tony, Nancy, to come together and really bring many, many different perspectives together in the pursuit of a treatment, a cure, and ultimately a prevention for ME/CFS. So, thank you to all of you for making this happen today.

Dr. Avindra Nath: Thank you, again. Now the meeting is adjourned.

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